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Pathogenicity of *Fusariumoxysporum* on upland New Rice for Africa NERICA Varieties and its Management with Botanical Extracts

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Abstract:

Studies were carried out to investigate the pathogenicity of *Fusariumoxysporum* on nine Nerica rice cultivars (Nerica 1, 2, 4, 7, 8, 10, 11, 14, 15), in comparison with WAB 56/104 and OS6. In vitro and in vivo investigations were also conducted to evaluate the crude extracts of *Azadirachta indica*, *Moringaoleifera* and *Vernoniaamygdalina* for the control of the fungus. Rice seeds were planted in sterilized soil and inoculated with (2.3×10^6) spores of *F. oxysporum* four weeks after planting. The plants were arranged in Completely Randomized Design (CRD) and replicated six times. Susceptible OS6 rice cultivar was planted in 5 kg pots filled with sterile top soil and inoculated with spores of *F. oxysporum*. Water extracts of the botanicals were then applied into the soil after 24 hours. It was arranged in Completely Randomized Design and replicated four times. Data were collected as above. Extracts of botanicals were applied at 25, 50 and 75 % concentrations respectively in vitro on Potato Dextrose Agar (PDA) and observed for percentage fungal growth inhibition. *Fusariumoxysporum* significantly ($P \leq 0.05$) reduced plant height, number of leaves and number of tillers in the inoculated plants compared to the uninoculated plants. Nerica 1, 7, 8, WAB 56/104 and OS6 were found to be susceptible while Nerica 2, 4, 10, 11, 14, and 15 showed levels of resistance. At 75 % botanical concentration, leaf chlorosis ranged between 1-10 %, while at 25 % concentration it ranged between 11-35 %. Leaf extracts were significantly more effective in reducing the radial growth of the pathogen at higher concentrations. Bitter leaf extracts (75 %), Neem extracts (75 %) and Moringa extracts (75 %) inhibited radial growth of *F. oxysporum* by 99.3, 93.5 and 81.1 % respectively. *Fusariumoxysporum* was confirmed to be pathogenic on NERICA rice; therefore, adequate control measures such as use of antagonistic botanical extracts can be recommended.

Keywords: *Oryza spp.*, root rot, *Vernoniaamygdalina*, *Moringaoleifera*, *Azadirachta indica*

1. Introduction

Rice, *Oryzasativa* is an important annual crop in Nigeria. It belongs to the family poaceae. It is one of the major staples which can provide a nations population with the nationally required food security minimum of 2400 calories per person per day (FAO 2000). The average Nigerian consumes 24.8 kg of rice per year, representing 9% of annual calorie intake (IRRI, 2001).

Due to its increasing contribution to the per capital calorie consumption of Nigerians, the demand for rice has been increasing at a much faster rate than domestic production and more than in any other African country since mid-1970s (FAO, 2001). In 2012, Nigeria produces 4.8 mt of rice (FAO, 2012). In spite of this Nigeria is still unable to meet local demand. The annual rice supply gap is being bridge by importation as Nigeria spends over ₦356b on yearly importation of rice. Nigeria however has huge but untapped potential for rice production and processing. According to the National Rice Development Strategy (NRDS 2010) only 39% of 4.6 million hectares of land suitable for rice production is under cultivation; less than 50,000 hectares of the estimated 3.14 million hectares of irrigable land is under rice irrigation; and on the average, less than half of the total amount of rice paddies in the country is processed. Constraints to rice production in Nigeria include: High input costs, policy instability; abiotic factors such as flooding, drought, soil fertility. The major biotic factors are problems of pests and diseases. The major rice diseases include: rice blast, bacterial leaf blight and rice yellow mottle virus. However, *Fusariumoxysporum* is a likely threat to the biotic factors affecting rice production in Nigeria. Prabhu and Bedendo (1983) reported that *F. oxysporum* caused basal node rot of rice in Brazil. Otokito and Umechuruba (2003) also isolated *Fusarium* from rice seeds and seedlings from rice nurseries in Bayelsa.

F. Oxysporum is one of the most economically important fungi worldwide (Robert *et al.*, 2001). In the United States, Fusarium wilt severely limited the production of cotton, causing losses of 109, 000 bales in 2004 (Blasingame and Patel 2005). Tomato producers also suffered immense losses due to the disease in many countries of the world (Walker, 1971, Volin and Jones, 1982).

Diseases management practices include; cultural practices, host plant resistance, chemical control, biological control and integrated pest management approach.

The use of fungicides (chemical control) is a very effective control option. However, the long term effects on human health, effect on the natural environment, risk of pathogen resistance to fungicide usage and the quality of agricultural products are of serious concern to all. The World Health Organization estimated that about 200,000 people die each year from pesticide poisoning and at least 3,000,000 people suffer acute health effect GOAN (1999). These associated risks have stimulated the interest in developing products from natural sources. Nature herself has offered array of plants with antibiotic properties. Such are *Azadirachtaindica*, *Moringaoleifera*, *Vernoniaamygdalina* and a host of others GOAN (1999). They are known for their rapid biodegradability and environmental friendly properties. Therefore the objectives of this study were: to evaluate the pathogenicity of *Fusariumoxysporum* on rice and to evaluate the efficacy of some plant extracts on the control of *F. oxysporum* on rice

2. Materials and Methods

Nine Nerica varieties (1, 2, 4, 5, 7, 8, 10, 11, 14) and WAB 56/104 and OS6 were collected from the Africa Rice Centre (former West African Rice Development Association) Ibadan.

Plant extracts were obtained from fresh leaves of *Vernoniaamygdalina*, *Moringaoleifera* and *Azadirachtaindica*. Fresh leaves were collected and washed in sterile distilled water and allowed to dry under shade until crisp. They were made into powdered form by milling with a dry blender. Ten grammes powder sample was then put into in separate conical flasks and 100 ml distilled water was added and was left to boil in a gallenkamp water bath at 80°C for 20 minutes and was allowed to cool (Onalo, 1999). The extracts were sieved through Whatman No 1 filter paper in a funnel. The filtrate obtained was taken to be the stock extract. Concentrations of 1:3 (25 percent), 1:1 (50 percent), and 3:1 (75 percent) stock to distilled water were prepared.

2.1. Evaluation of Rice Cultivars for Their Reaction to *Fusariumoxysporum*

Three rice seeds were planted in polythene bags filled with sterile top soil (5000 cm³). Eleven rice cultivars (Nerica 1, 2, 4, 5, 7, 8, 10, 11, 14, WAB56/104 and OS6) were planted. Four weeks after planting, *Fusariumoxysporum* at a spore concentration of 2.3 x 10⁶ per milliliter were inoculated on the rice cultivars. This was done by introducing 1 ml of the inoculum suspension into holes at the base of the plants with the aid of a syringe.

The experiment was arranged in a Completely Randomised Design with six replicates. Data were collected on plant height, number of leaves and number of tillers. Diseases symptoms were also scored using a scale of 0-5. 0 = No damage on the leaf; 1 = 1- 10% damage; 2 = 11- 20% damage; 3 = 21- 35% damage; 4 = 36- 60% damage; 5= 60% damage and above. A disease index of root lesion formed by the fungi was determined by the length and severity of the lesion (McGee and Kellock 1974) using the following scale; 0 = no visible lesion; 1 = lesion 1mm long; 2 = lesion 1 to 5mm long, brown; 3 = lesion longer than 5mm, dark brown; 4 = root totally infected and brown; 5 = root destroyed and plant dying or dead. Root lesion length was measured with the aid of thread which was later measured with a meter rule. Height of plant was recorded with the aid of meter rule; number of leaves was counted on each plant. Chlorosis and necrosis scores were recorded on two leaves per tiller.

2.2. In Vivo Evaluation of Plant Extracts

Rice seed was planted in polythene bags filled with sterile top soil (5000 cm³). Spore suspension (2.3 x 10⁶) of *F. oxysporum* was inoculated into the soil with syringe. Botanicals were also applied unto the soil. Data were collected weekly as for the pathogenicity test above.

2.3. In Vitro Evaluation of Extracts

With the aid of 5 mm diameter cork borer mycelia disc were cut from 5 days old culture of *F. oxysporum* and *B. theobromae*. Each of the mycelia discs was aseptically transferred with the aid of flame sterilized inoculating needle to the centre of solidified PDA-extract medium contained in Petri dishes, the bottom of which have been previously marked with two perpendicular lines passing through the centre.

Plant extracts of 240 ml, 160 ml and 80 ml were mixed with 80 ml, 160 ml and 240 ml of sterilized PDA to make 75, 50 and 25 % concentrations respectively. Plain PDA served as control. The experiment were arranged in a Completely Randomised Design and replicated four times. It was incubated on laboratory benches at room temperature. Plates without extracts served as control. Measurement of colony diameters was taken in two directions along the perpendicular lines at 48 hours interval and terminated on the seventh day of incubation. The mean radial growth was calculated for each treatment by finding the average of the two measurements taken from the perpendicular lines. The percentage growth inhibition was calculated according to Pandey *et al.*, (1982).

Growth inhibition (%) = $\frac{DC - DT}{DC} \times 100$

DC x100

DC = Average diameter of the control

DT = Average diameter of fungal colony with treatment

3. Results

3.1. Plant Height, Number of Leaves and Number of Tillers of 11 Rice Cultivars as Affected by *Fusariumoxysporum*

Nerica 15 was the tallest plant, but it was not significantly ($P \leq 0.05$) different from Nerica 10, Nerica 4 and Nerica 14 as shown in Table 1. Rice cultivar OS6 was the shortest. Nerica 2 had the highest number of leaves but it was not significantly ($P \leq 0.05$) different from Nerica 14. Nerica 10 and Nerica 4 followed in that order. Cultivars OS6 and WAB 56/104 had the least number of leaves. Nerica 2 had significantly ($P \leq 0.05$) higher number of tillers compared to other cultivars. This was followed by Nerica 4, which was not significantly ($P \leq 0.05$) different from Nerica 11. Cultivar OS6 had the least number of tillers.

Cultivars	Plant height (cm)	No of leaves	No of tillers
Nerica 15	26.7a	9.6e	4.2bc
Nerica 10	26.3a	12.7b	4.1bc
Nerica 4	26.2a	11.2c	4.7b
Nerica 14	26.1ab	13.1a	4.2bc
Nerica 2	25.9b	13.9a	6.0a
Nerica 11	25.4b	10.4d	4.6b
Nerica 1	23.7bc	8.6f	2.3f
Nerica 8	23.3bc	8.6f	2.7f
Nerica 7	22.2c	8.3fg	3.4d
WAB56/104	15.4 d	6.70h	3.2de
OS6	10.2e	6.0h	1.9g

Table 1: Plant height, number of leaves and number of tillers of 11 rice cultivars, As affected by *Fusariumoxysporum*

Values are means of six replicates per treatment

Means followed by the same alphabets within a column are not significantly different at 5 % level of probability using DMRT.

3.2. Effects of *Fusariumoxysporum* Chlorosis, Necrosis and Root Lesion Severity of Eleven Rice Cultivars.

Nerica 14 and 15 had significantly ($p \leq 0.05$) lowest leaf chlorosis severity compared to other cultivars (Table 2). Cultivar OS6 had the highest leaf chlorosis severity. This was followed by Nerica 1, 7 and 8. Nerica 7 had the highest leaf necrosis but was not significantly ($p \leq 0.05$) different from Nerica 1, OS6 and WAB 56/104. However, Nerica 4, 10 and 15 had the least leaf necrosis. Cultivar OS6 had the highest root lesion severity; this was followed by 1, 7 and 8 which were statistically at par. On the other hand, Nerica 2, 10, 11 and 14 had the least root lesion severity.

Cultivars	chlorosis	Leaf Necrosis severity	Root lesion severity
Nerica 11	2.3de	2.2d	2.0f
Nerica 4	2.7d	1.1e	2.3d
Nerica 10	1.9g	1.1e	2.2ef
WAB56/104	3.1b	3.3a	2.5c
Nerica 15	1.3h	1.1e	2.7c
Nerica 14	1.3h	1.2e	2.0 f
Nerica 1	2.9bc	3 .6a	3.5b
Nerica 8	3.2b	2.6bc	3.3b
OS6	4.9a	3.6a	5.1a
Nerica 2	2.1f	2.7b	2.1f
Nerica 7	2.9bc	3.7a	3.5b

Table 2: Effects of *Fusariumoxysporum* chlorosis, necrosis and root lesion severity of eleven rice cultivar

Values are means of six replicates per treatment Means followed by the same alphabets within a column are not significantly different at 5 % level of probability using DMRT

3.3. Effects Of *Fusariumoxysporum* On Plant Height Of OS6 Rice Cultivar Treated With Three Concentrations Of Botanicals.

The effectiveness of the plant extracts were concentration dependent (Table 3). All the plants inoculated with the pathogen treated with the plant extracts at 75 % concentration had significantly ($p \leq 0.05$) higher plant height compared to the plants treated with plant extracts at lower concentration (25 and 50 %). Rice inoculated with *F.oxysporum* treated with bitter leaf extract was the shortest.

Cultivars + Pathogens	25, 000mgkg ⁻¹	50, 000mgkg ⁻¹	75, 000mgkg ⁻¹
<i>F. oxysporum</i> + Bitter leaf	5.2ef (c)	8.3e (b)	14.1f (a)
<i>F. oxysporum</i> + Neem	7.4 d (c)	10.2d (b)	15.1e (a)
<i>F. oxysporum</i> + Moringa	8.1c (c)	10.1 (b)	14.1 (a)
Control	26.4 a (a)	24.7a (a)	25.8a (a)

Table 3: Effects *Fusariumoxysporum* on plant height of OS6 rice cultivar treated with three concentrations of botanicals.

Values are means of six replicates per treatment

Means followed by the same alphabets within a column are not significantly different at 5 % level of probability using DMRT

3.4. Effects of *Fusariumoxysporum* on Number of Leaves of OS6 Rice Cultivar Treated with Three Concentrations of Botanicals.

The plant extracts were more effective at higher concentration in controlling the pathogens, except for plants inoculated with *F. oxysporum*, treated with neem extracts (25 and 50 % concentration) which had significantly ($p \leq 0.05$) the same number of leaves (Table 4). Uninoculated rice cultivar had significantly ($p \leq 0.05$) higher number of leaves compare to rice inoculated with pathogens and treated with plant extracts at all concentrations. Rice inoculated with *F.oxysporum* treated with moringa had the least number of leaves.

Fungi + Botanicals	25, 000mgkg ⁻¹	50, 000mgkg ⁻¹	75, 000mgkg ⁻¹
<i>F. oxysporum</i> + Bitter leaf	4.7c (c)	7.3c (b)	13.3e (a)
<i>F.oxysporum</i> + Neem	8.6b (b)	9.3b (b)	14.1d (a)
<i>F. oxysporum</i> + Moringa	8.1b (c)	10.3b (b)	10.1f (a)
Control	17.2 a (a)	16.9 a (a)	15.7a (a)

Table 4: Effects of *Fusariumoxysporum* on number of leaves of rice cultivar OS6 treated with three concentrations of botanicals.

Means followed by the same alphabets within a column are not significantly different. Means in parenthesis along the row are not significantly different at 5 % level of probability using DMRT

3.5. Effects of *Fusariumoxysporum* on Number of Tillers of OS6 Rice Cultivar Treated with Three Concentrations of Botanicals.

Uninoculated rice cultivar had significantly ($p \leq 0.05$) higher number of tillers compare to rice inoculated with either pathogens and treated with plant extracts at all concentrations (Table 5). At 50 % concentration, rice inoculated with *F.oxysporum* treated with moringa had the highest number of tillers. The effectiveness of the plant extracts were also concentration dependent, except in very few cases.

Fungi + Botanicals	25, 000mgkg ⁻¹	50, 000mgkg ⁻¹	75, 000mgkg ⁻¹
<i>F. oxysporum</i> + Bitter leaf	2.8d (b)	2.4d (b)	4.4d (a)
<i>F.oxysporum</i> + Neem	2.6d (b)	3.3c (a)	4.7d (a)
<i>F. oxysporum</i> + Moringa	3.9c (b)	4.7b (a)	4.3d (a)
Control	8.3a (a)	9.4a (a)	8.9a (a)

Table 5: Effects of *Fusariumoxysporum* on number of tillers of OS6 rice cultivar treated with three concentrations of botanicals.

Means followed by the same alphabets within a column are not significantly different. Means in parenthesis along the row are not significantly different at 5 % level of probability using DMRT

3.6. Effects of *Fusariumoxysporum* on Chlorosis of OS6 Rice Cultivar Treated with Three Concentrations of Botanicals.

Uninoculated rice cultivar had the least leaf chlorosis severity but was not significantly ($p \leq 0.05$) different from rice inoculated with *F.oxysporum* treated with neem (75 %) (Table 6). Rice inoculated with *F.oxysporum* treated with moringa had the highest leaf chlorosis severity. The plant extracts were more effective at higher concentration.

Fungi + Botanicals	25, 000mgkg ⁻¹	50, 000mgkg ⁻¹	75, 000mgkg ⁻¹
<i>F. oxysporum</i> + Bitter leaf	2.9c (a)	2.2b (b)	1.7a (c)
<i>F.oxysporum</i> + Neem	3.2b (a)	1.9c (b)	0.5c (c)
<i>F. oxysporum</i> + Moringa	4.1a (a)	1.8c (b)	0.8b (c)
Control	0.5e (a)	0.4f (a)	0.4c (a)

Table 6: Effects of *Fusariumoxysporum* on chlorosis of OS6 rice cultivar treated with three concentrations of botanicals.

Means followed by the same alphabets within a column are not significantly different. Means in parenthesis along the row are not significantly different at 5 % level of probability using DMRT.

3.7. Effects of *Fusariumoxysporum* on Necrosis of Rice Cultivar OS6 Treated with Three Concentrations of Botanicals.

Uninoculated rice cultivar had the least leaf chlorosis severity, but was not significantly ($p \leq 0.05$) different from rice inoculate with *F.oxysporum* treated with neem (75 %) as shown in Table 7. At 50 % concentration, rice inoculated with *F.oxysporum* treated with neem extract had the least leaf necrosis severity, while inoculated with the same pathogen, treated with moringa had the highest leaf necrosis severity. At 25 % concentration, rice inoculated with *F.oxysporum* treated with moringa extracts had the highest leaf necrosis severity. The plant extracts were more effective at higher concentration.

Fungi + Botanicals	25, 000mgkg ⁻¹	50, 000mgkg ⁻¹	75, 000mgkg ⁻¹
<i>F. oxysporum</i> + Bitter leaf	2.6b (a)	1.3b (b)	0.9b (b)
<i>F.oxysporum</i> + Neem	1.9c (a)	0.8c (b)	0.3c (c)
<i>F. oxysporum</i> + Moringa	3.5a (a)	1.9a (b)	1.4a (c)
Control	0.3e (a)	0.2d (a)	0.2c (a)

Table 7: Effects of *Fusariumoxysporum* on necrosis of OS6 rice cultivar treated with three concentrations of botanicals.

Means followed by the same alphabets within a column are not significantly different. Means in parenthesis along the row are not significantly different at 5 % level of probability using DMRT

3.8. Effects of *Fusariumoxysporum* on Root Lesion of Rice Cultivar OS6 Treated with Three Concentrations of Botanicals.

Uninoculated rice cultivar had the least root lesion severity (Table 8). Rice inoculated with *F.oxysporum* treated with bitter leaf extracts had highest root lesion severity. At 50 % concentration, rice inoculated with *F.oxysporum* treated with neem extract had the least root lesion severity, while rice inoculated with the same pathogen, treated with moringa had the highest root lesion severity. At 25 % concentration, rice inoculated with *F.oxysporum* treated with neem extracts had the least root lesion severity.

Fungi + Botanicals	25, 000mgkg ⁻¹	50, 000mgkg ⁻¹	75, 000mgkg ⁻¹
<i>F. oxysporum</i> + Bitter leaf	3.8c (a)	3.0c (b)	3.6a (a b)
<i>F.oxysporum</i> + Neem	3.0d (a)	2.7d (a)	3.0b (a)
<i>F. oxysporum</i> + Moringa	4.2b (a)	3.9a (b)	2.8c (c)
Control	0.4e (a)	0.3e (a)	0.5e (a)

Table 8: Effects of *Fusariumoxysporum* on root lesion of OS6 rice cultivar treated with three concentrations of botanicals.

Means followed by the same alphabets within a column are not significantly different. Means in parenthesis along the row are not significantly different at 5 % level of probability using DMRT

3.9. Percentage Growth Inhibition of *Fusariumoxysporum* by Three Concentration of Plant Extracts (%).

All the three plant extracts tested significantly ($p \leq 0.05$) inhibit mycelia growth of *F.oxysporum* *in vitro* (Table 9). At 25 % concentrations inhibition of *F. oxysporum* ranged from 10.3- 32.7 %. At 50 % concentrations inhibition of *F. oxysporum* ranged from 46.9-65 %, while at 75 % concentrations inhibition of *F. oxysporum* ranged from 81.1- 99.3 %.. Bitter leaf extracts at 75 % concentration gave the highest inhibition rate (99.3 %).

Fungi + Botanicals	25, 000mgkg ⁻¹	50, 000mgkg ⁻¹	75, 000mgkg ⁻¹
<i>F. oxysporum</i> + Bitter leaf	26.7c (c)	65.6a (b)	99.3a (a)
<i>F.oxysporum</i> + Neem	32.7b (a)	59.3b (b)	93.5b (c)
<i>F. oxysporum</i> + Moringa	26.7c (a)	46.9d (b)	81.1c (c)
Control	10.3 d (a)	12.7e (a)	14.7d (a)

Table 9: Percentage growth inhibition of *Fusariumoxysporum* by three concentration of plant extracts (%).

Means followed by the same alphabets within a column are not significantly different. Means in parenthesis along the row are not significantly different at 5 % level of probability using DMRT

4. Discussion

The study showed that *Fusariumoxysporum* is pathogenic on rice. This agreed with the work of Prabhu and Bedendo (1983). They reported that *F.oxysporum* caused basal node rot of rice in Brazil. Also, Otokito and Umechuruba (2003) isolated *F. oxysporum* from rice seeds and seedlings in Bayelsa. *Fusariumoxysporum* has also been isolated from tomato, cowpea and muskmelon (Raabeet al.,

1981, Ojuderie, 2002). Rice inoculated with *F. oxysporum* showed symptoms of wilting, chlorosis and necrosis, this was reported by Raabe *et al* (1981), who found the fungus causing chlorosis and wilting on tomato and soyabean. Agrios (2005) also reported that *F. oxysporum* caused symptoms such as stunting, defoliation, marginal necrosis, and chlorosis in plants. Among the eleven rice cultivars evaluated, Nerica 2, 4, 10, 11, 14, and 15 showed levels of resistance. Yacouba *et al.*, 2008 reported that Nerica 15 is resistant to rice blast disease. Nerica 1, 7, 8, WAB 56/104 and OS6 were susceptible. Oyetunji (2009) reported severe rot in rice root inoculated with *Botryodiplodia theobromae*. This work revealed that fungitoxic compounds are present in *Moringa oleifera*, *Azadirachta indica* and *Vernonia amygdalina* since they were able to suppress the growth of microorganisms tested both *in vivo* and *in vitro*. This agreed with earlier reports by some workers on the effects of these plants extracts on different pathogens

Amadioha and Obi (1998) demonstrated the fungitoxic activity of seed extract of *Azadirachta indica* (neem) and *Xylopiiaethiopia* against the anthraconose fungus (*Collectotrichum lindemuthianum*) of cowpea. Agbenin and Marley (2006) reported that fresh neem leaf extracts reduced mycelial growth of *F. oxysporum* f.sp. *Lycopersici*. Shervinet *et al.*, 2011 reported that neem seed powder caused increase growth of tomato plant. Dwivedi and Sangeeta (2014) reported that *Moringa oleifera* (bark) showed antifungal activity against *F. oxysporum*. Adandonon *et al.*, (2006) also found moringa to contain antimicrobial properties. Enikuomehinet *al.*, 1998 reported the antifungal activities of *Vernonia amygdalina*.

5. Conclusion and Recommendation

Fusarium oxysporum was found to be pathogenic on rice by inducing, chlorosis, necrosis wilting and root lesion. It was also established that the test plants *Vernonia amygdalina*, *Azadirachta indica* and *Moringa oleifera* possess antifungal substances toxic to the test pathogen both *in vitro* and *in vivo*. However the plant extracts were more efficient at higher concentration (75 %). It is recommended that further studies should be conducted to ascertain the yield losses attributed to the pathogen on rice. Also other means of extraction of the plant extracts other than crude extracts should also be explored.

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