Jewel Journal of Scientific Research (JJSR) 7(1): 70–78, 2022
©Federal University of Kashere-Nigeria (Print ISSN: 2384 – 6267, Online ISSN 2756-651X)
jjsr.fukashere.edu.ng

Fungal Diversity in selected Cultivated Farmland within the University of Ilorin Campus

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Abstract
This study was carried out to determine the fungal biota composition of selected agricultural soils and to determine the soil p^H level and organic matter content that supports the observed fungal community in the soils within the University of Ilorin campus. Serial dilution technique was used to determine the fungal colony count of the sampled soils. Soil p^H was determined with the use of p^H meter and organic matter content of the soil was determined using the Walky Black procedure. Nine species of fungi belonging to seven genera were isolated from all the sampled soils. The fungi were identified as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceous*, *Botryodiplodia theobromae*, *Cladosporium* sp., *Fusarium verticilloides*, *Penicillium* sp., *Phomopsis* sp. and *Pythium* sp. There was no significant difference ($p>0.05$) in the population of the isolated fungi in all the sampled soils except for *Phomopsis* sp. in soybean cultivated soil samples and *Fusarium* sp. in soils sampled from zone C. The results also showed no significant difference ($p>0.05$) in the organic matter content of the soils. The sampled soils were generally slightly acidic with p^H values ranging between 6.03 and 6.88. Significant differences ($p<0.05$) only existed between the p^H values of cassava cultivated soils obtained from the different zones. These organic matter content and the p^H values of the soils were found to be within the range suitable for the survival of the fungal community in the study area.

Keywords: soil pH, organic matter, mycoflora, distribution

Received: 22nd Dec., 2021 *Accepted:* 24th Mar., 2022 *Published Online:* 7th April, 2022

Introduction

Fungi are an important part of the soil ecosystem where they play diverse roles. They are particularly known to take part in decomposition of organic matter and the recycling of soil nutrients to make them available to plants (Val-Moraes *et al*., 2013). In this way they contribute to soil fertility and alleviation of soil degradation. Fertile soil supports growth of plants, in-turn these plants produce vital needs to humans like food, clothing, furniture, medicine, etc. as well, animals and insects obtain food from the plants and plant products.

Other microbes like bacteria and algae also cohabits the soil with fungi and they all help in maintaining environmental balance (retention of moisture, decay of dead bodies of animals, plants etc., scavenging of waste and other toxic chemicals including plastic). According to Tedersoo (2014), there are about 80,500 fungal operational taxonomic units in soils worldwide. Soil fungal diversity in any given environment is however affected by the local environmental conditions (Tardy

et al, 2015), including the chemical and physical soil characteristics, which determine to a great extent the composition of extant fungal communities (Requena *et al*, 2001; Rousk *et al*., 2010).

Soil-borne diseases of ancient and modern agricultural crops have always had some impact on growth and productivity. Soil-borne diseases are very critical in reducing the yield potential of improved cultivars in several agricultural crops. Nearly 25% of loss in yield in developing countries is due to soil-borne fungal diseases (Janvier *et al*, 2007). Often these diseases are very difficult to manage due to their high heterogeneous incidence and lack of knowledge on the epidemiological aspects of soil-borne pathogens. To reduce the disease incidence and increase production of food for the increasing world population, adequate control measures need to be taken (Gohel *et al*. 2006). The effective control and management of soil-borne disease is possible only if detailed study of survival, dissemination of soil-borne pathogens, the effect of environmental conditions, role of cultural practices and host resistance and susceptibility are properly understood.

This study therefore aims at understanding the microbial composition of the soil within the University of Ilorin campus and to understand the chemical status of the soil with a view to understanding the relationship between the observed composition of the fungal community and their chemical status. The specific objectives of the study were to isolate and identify fungal community contained in the soil of selected farmland within the University of Ilorin campus and to determine the soil chemical characteristics (pH and organic matter content) supporting the microbial community in the sampled soils.

Materials and Methods

Sampling site and collection of soil samples

Sampling of soil was carried out within the University of Ilorin. The university is located at the north-central part of Nigeria (8.4928° N, 4.5962° E). The sampled areas were divided into 3 zones (A, B and C). Zone A was located on the Teaching and Research Farm of the Faculty of Agriculture. Zone B was located on the Biological garden of the Faculty of Life Sciences while Zone C was located around the senior staff quarters. The collection of soil samples was done on randomly selected soybean, maize and cassava farms located at each zone. Sample collection was done between August and September in the year 2020 in accordance with the protocol described by Carrasco *et al*, 2002. The distribution of farms in each zone is as shown in Table 1 below.

The soil samples collected were taken to the Laboratory of the Department of Crop Protection Faculty of Agriculture, University of Ilorin where stones and plant roots were removed by sieving in 2mm mesh sieve followed by air-drying within three days. A portion of each of the soil samples was used to determine soil characteristics while the other portion was used for isolation of the fungal biota.

Table 1: Soil sampling zones

Zones	Number of soybean farms found	Number of soybean farms sampled	Number of maize farms found	Number of maize farms sampled	Number of cassava farms found	Number of cassava farms sampled
Zone A	7	5	10	5	10	5
Zone B	6	5	7	5	9	5
Zone C	10	5	12	5	10	5
Total	**23**	**15**	**29**	**15**	**29**	**15**

Soil Analysis

Three different types of soil analysis were carried out on each soil sample, these include soil pH, organic matter and isolation of fungi.

Soil pH

For each soil sample, 10g of preserved soil sample was weighed into 50ml beakers. 25ml of distilled water were added into each beaker containing the soil sample, this was then stirred continuously for 10 minutes and allowed to stand for 30 minutes. Stirring was again done for 2 minutes with the use of a glass rod after which the mixture was allowed to stand for 15munites. Thereafter, an electrode pH meter was inserted into the partly settled suspension. The pH record of the suspension of all the soil samples after standardizing the pH meter with a buffer solution of known pH (buffer 4 and buffer 9 respectively) were taken. The mixtures were also allowed to settle for one hour in order to measure the conductivity of the supernatant.

Organic matter

For each preserved soil sample, 1g was weighed into a 500ml Erlenmeyer flask; ten milliliters (10ml) of 1N potassium dichromate was added with the use of pipette and was swirled gently to disperse the soil. 10ml of concentrated sulphuric acid (H_2SO_4) was thereafter added to the mixture and allowed to stand for 30 minutes and this was followed by the addition of 100ml of dilled water. Thereafter, 3 – 4 drops of Ferroin indicator was added and titrated with 0.2N ferrous ammonium sulphate solution (as the end-point approached, the solution took a greenish cast and subsequently changed to dark green). At this point, ferrous ammonium sulphate was added drop by drop until the color changed sharply from blue to maroon (colour in reflected light against a white background). The same titration procedure was carried out, but with soil to standardize the potassium dichromate. The experiment was duplicated and the values obtained were used to determine the following parameters:

$$\text{Percentage of organic carbon} = \frac{\text{blank (B)} - \text{titre (T)} * 0.03 * \text{correcting factor} * 100}{\text{Weight of the sample}}$$

$$\text{NF} = \frac{\text{(Concentration} * \text{volume) of potassium dichromate used}}{\text{Titre value of the blank}}$$

Percentage of organic matter = percentage of organic carbon X Walkly constant.

Where:

Blank = titre value of the blank

T = titre value of the sample

NF = Normality of Potassium dichromate

Concentration of potassium dichromate = 1

Volume of potassium dichromate = 5ml

Walkly constant = 1.7241

Correcting factor = 1.33

Isolation of fungi

Soil dilution plate method was used to isolate the fungi contained in the soil samples. 90mm of sterile distilled water was measured into 10g of each soil sample in 250ml Erlenmeyer flask and the mixture was shaken thoroughly to produce a mixture. One milliliter of the resulting suspension was pipetted into 9ml of sterile water in a glass test tube to make 10^{-2} dilution. The procedure was repeated to make 10^{-3} dilution. One milliliter of the 10^{-3} dilution was pipetted into solidified PDA medium already amended with streptomycin to suppress bacteria growth. 1ml suspension of the 10^{-3} dilution was spread evenly on the surface of the PDA plate using bent glass rod and the experiment was replicated 3 times. The plates were then incubated at 25^0C for 5 – 7 days. The isolated fungi were then enumerated on the basis of their morphological differences on the plate.

Preparation of Pure cultures of the isolates

Hyphae or spores were transferred aseptically into fresh sterile media with an ethanol flamed inoculating needle to prepare the pure culture from each of the fungal isolate. The plates were incubated for seven days at 25^0 C $\pm$ 2^0 C. The pure cultures were used for the identification of the fungal isolates.

Identification of the Isolates

Morphological characteristics of the isolated fungi were observed. Temporary slides were prepared for each of the isolates. The slides were then observed under a compound microscope. The morphological and microscopic features were recorded. These features were matched with those described

in standard references for identification. (The identities of the isolates were confirmed at The Plant Pathology Laboratories of The International Institute for Tropical Agriculture (IITA) Ibadan Nigeria).

Experimental Design, Data collection and Statistical analysis

The experiment was laid out in a Completely Randomized Design (CRD). Data obtained from soil analysis and isolation of fungi was subjected to 1- way Analysis of Variance (ANOVA) using the IBM SPSS version 21 Statistical Package. Mean separation was done at 5% level of significance. Tests were The result of the organic matter content of the sampled soils is as shown in Tables 3. There is no significant difference ($p>0.05$) in the organic matter content of all the sampled soils. The percentage organic matter content adjusted for all pairwise comparisons using the Bonferroni correction.

Results and Discussion

The result of the soil analysis for p^H is as shown in Table 2, below. There is no significant difference ($p>0.05$) in the p^H values of the surveyed maize and soybean soils in all the zones. There was however a significant difference ($p<0.05$) in the p^H values of cassava soils in the different zones. All the soils were slightly acidic with p^H values ranging from 6.03 on soybean soil to 6.88 on cassava soil.

of sampled maize grown soils ranged from 0.7 – 3.14, while that of soybean ranged from 0.59 – 1.44 and of cassava ranged from 0.51 – 1.56.

Table 2: p^H level of the sampled soils

Crops	Zone A	Zone B	Zone C
Cassava	6.61a±0.08	6.71a±0.01	6.88b±0.08
Maize	6.59a±0.10	6.56a±0.05	6.64a±0.03
Soybean	6.23a±0.15	6.03a±0.27	6.05a±0.29

Values in the same row not sharing the same letters are significantly different at p< .05 in the two-sided test of equality for column means. Tests assume equal variances.[1] Tests are adjusted for all pairwise comparisons using the Bonferroni correction.

Table 3: Organic matter content of the sampled soils

Crops	Zone A	Zone B	Zone C
Cassava	1.15a±0.38	1.04a±0.26	1.19a±0.23
Maize	1.39a±0.51	2.44a±0.72	1.62a±0.61
Soybean	1.27a±0.26	1.02a±0.33	1.25a±0.32

Values in the same row not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column means. Tests assume equal variances.[1] Tests are adjusted for all pairwise comparisons using the Bonferroni correction.

Nine fungal species belonging to seven genera were isolated from the sampled soils. The isolated fungi include *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceous, Botryodiplodia theobromae, Cladosporium* sp., *Fusarium verticilloides, Penicillium* sp., *Phomopsis* sp. and *Pythium* sp. (Fig 1a-i).

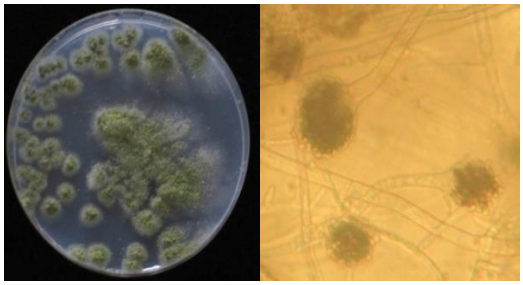


Plate1a: *Aspergillus flavus*

Morphological description. Colonies on PDA contained a diameter of 3-5 cm within 7 days, usually consisting of dense felt yellow-green conidiophores. Conidial heads typically radiate, later splitting into several loose columns, yellow-green becoming dark yellow-green.

Microscopic description. Conidiophores hyaline coarsely roughened, up to 1.0 mm (some isolates up to 2.5mm) in length phialides borne directly on the vesicle or metulae, 6-10 × 4.0-5.5um, Metulae 6.5-10 ×3.5um Conidia globose to subglobose

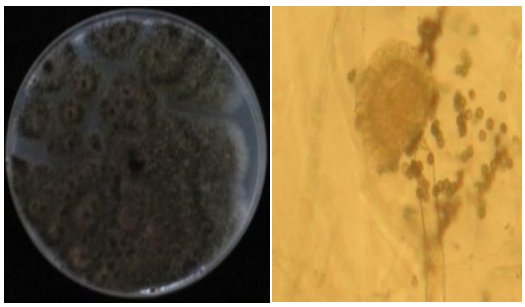


Plate 1b: *Aspergillus niger*

Morphological description. Colonies on PDA contained a diameter of 4-5 cm within 7 days, usually consisting of compact white or yellow basal felt with a dense layer of dark brown to black conidiophores.

Microscopic description. Conidial heads, black radiate, tending to split into columns with age. Conidiophores stipes smooth walled hyaline but also in brown color. Phialides borne on metulae, 7.0-09 × 3.5um, and hyaline to brown often, 15-25 × 4, 5-6.0um. Conidia globose to subglobose

Plate 1c: *Aspergillus ochraceous*

Morphological description. Colonies on PDA contained a diameter of 3-5 cm within 7 days, usually consisting of dense felt yellow-green conidiophores. Conidial heads typically radiate, later splitting into several loose columns, yellow-green becoming dark yellow-green.

Microscopic description. Conidiophores hyaline coarsely roughened, up to 1.0 mm (some isolates up to 2.5mm) in length phialides borne directly on the vesicle or metulae, 6-10 × 4.0-5.5um, Metulae 6.5-10 ×3.5um Conidia globose to subglobose

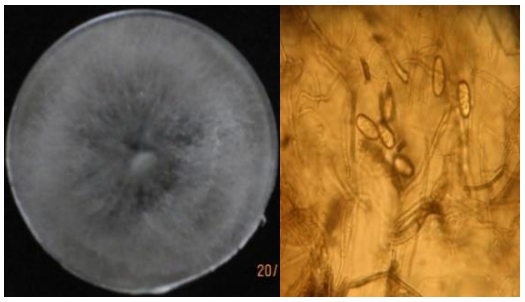


Plate 1d: *Botryodiplodia theobromae*

Morphological description. The organism showed vegetative growth, fluffy colony growth, they exhibited an ir-regular growth pattern. The colony color of isolates ranges from dull white, cottony white changing to black

Microscopic description. The main features of this organism are the presence of pycnidial paraphyses and longitudinal striations on mature conidia

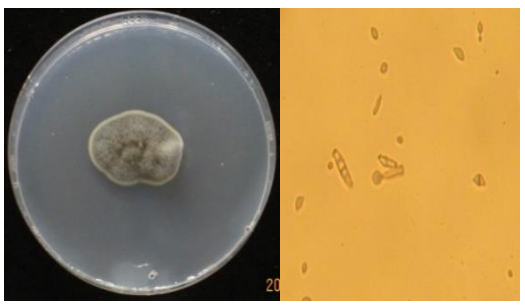
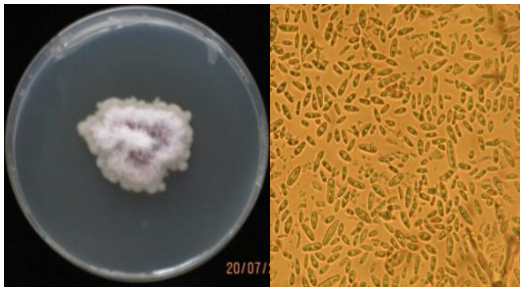


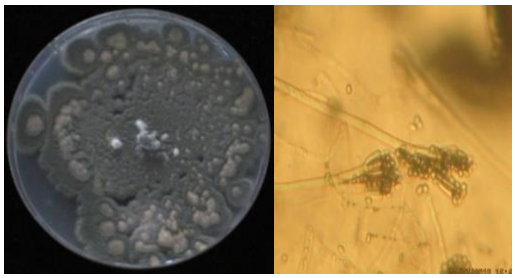
Plate 1e: *Cladosporium* sp.

Description. Conidiophores tall, dark, upright, branched variously near the apex, clustered or single, Conidia (blastopores) dark, 1 or 2 celled, variable in shape and size, ovoid to cylindrical and irregular, some typical lemon-shaped; often in simple or branched acropetalous chains;



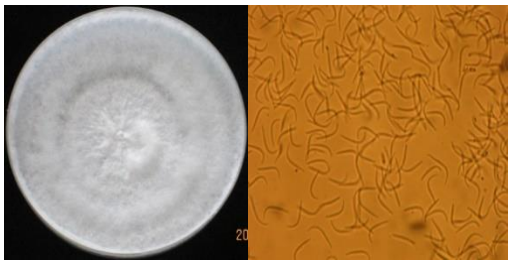
Description. Micro conidia abundant and primarily single-celled, oval to club-shaped. Macro conidia are present, though sometimes rare. Their appearance varies from slightly sickle-shaped to almost straight.

Plate 1f: ***Fusarium verticilloides***



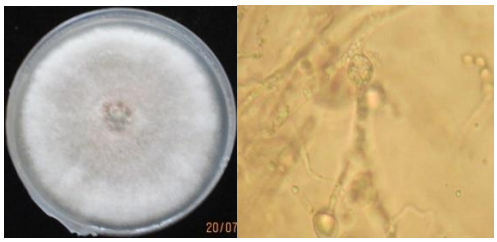
Description. Conidiophores arising from a single or less often synnemata branched near the apex, penicillate, ending in phialides: conidia hyaline, 1 celled, mostly globose or ovoid, in

Plate 1g: ***Penicillium*** **sp.**



Description. Pycnidia dark, ostiolate, immersed, erumpent, nearlyglobose, conidiophores simple; conidia hyaline, I celled, of two types, ovoid to flusoid (alpha) conidia, and filiform, curved or bent (beta) conidia

Plate 1h: ***Phomopsis*** **sp.**



Morphological description. Mycelium hyaline, well branched. Main hyphae up to 6–8 µm wide. Colonies on PDA are submerged and show a narrow chrysanthemal pattern. Average radial growth of the oomycete on PDA is 11 mm/day. It grows well in water on hemp-seed halves and produces asexual and sexual structures at room temperatures (18–25°C). **Microscopic description.** Sporangia are globose to somewhat cylindrical, oval, and at times peanut shaped mostly catenulate and intercalary; measuring 15–55 µm in diameter and up to 65 µm in length. These structures are densely granulated and the larger ones have a clear hyaline central zone which is sparsely granulated.

Plate 1i: ***Pythium*** **sp.**

The occurrence of the fungal isolates in the sampled soils of maize, soybean and cassava from the different zones is summarized in Table 4. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceous*, *Cladosporium sp., Fusarium verticillioides, Penicillium sp., Phythium* sp. were found in all the soils. *Phomospsis sp.* and *Botryoodiplodia theobromae* were not found on cassava and soybean soils.

Statistically, there was no significant difference ($p>0.05$) in the mean percentage

occurrence of the fungal isolates in cassava and maize cultivated soils but the mean percentage of *Phomopsis* sp. differed significantly ($p<0.05$) from other isolates in soybean cultivated soils (Table 5). With respect to the different zones, the result was similar to what was observed for the different crops with only *Fusarium* sp. isolate showing a significantly different population ($p<0.05$) from other isolates in Zone C (Table 6).

Table 4: Occurrence of Fungal isolates in sampled soils

Fungal Isolates	Cassava	Maize	Soybean
Aspergillus flavus	+	+	+
Aspergillus niger	+	+	+
Aspergillus ochraceous	+	+	+
Botryodiploidea sp.	_	+	_
Cladosporium sp.	+	+	+
Fusarium verticilloides	+	+	+
Penicillium sp.	+	+	+
Phomopsis sp.	_	+	_
Pythium sp.	+	+	+

Table 5: Incidence (%) of Fungi isolated from soils cultivated with different crops within the University of Ilorin Campus

Fungal Isolates	Cassava	Maize	Soybean
Aspergillus flavus	1.98a ± 1.00	2.27a ± 1.00	1.87a ± 0.79
Aspergillus niger	3.35a ± 1.21	2.87a ± 0.93	3.25a ± 0.86
Aspergillus ochraceous	0.91a ± 0.68	0.96a ± 0.65	0.87a ±0.50
Botryodiploidea sp.	0.46a ± 0.68	0.36a ± 0.54	0.50a ±0.59
Cladosporium sp.	0.30a ± 0.60	0.36a ±0.54	0.62a ±0.82
Fusarium verticilloides	2.13a ± 1.21	2.27a ±0.84	1.25a ±0.68
Penicillium sp.	1.22a ± 0.82	1.31a ±0.72	1.12a ±0.79
Phomopsis sp.	0.00a ± 0.00	0.12a ±0.36	0.62b ±0.59
Pythium sp.	0.76a ±0.72	0.60a ± 0.78	1.00a ±0.37

Values in the same row and subtable not sharing the same subscript are significantly different at $p<0.05$ in the two-sided test of equality for column means. Tests are adjusted for all pairwise comparisons using the Bonferroni correction.

Table 6: Incidence (%) of Fungi isolated from soil samples from the different sample zones within the University of Ilorin Campus

Fungal Isolates	Zone A	Zone B	Zone C
Aspergillus flavus	1.95a± 1.07	2.14a± 1.00	2.06a± 0.81
Aspergillus niger	3.17a± 0.37	3.61a± 1.04	2.61a± 0.97
Aspergillus ochraceous	1.22a± 0.37	0.80a± 0.60	0.69a± 0.65
Botryodiploidea sp.	0.12a ± 0.37	0.67a ± 0.63	0.55a ± 0.65
Cladosporium sp.	0.37a ± 0.78	0.54a ± 0.63	0.41a ± 0.62
Fusarium verticilloides	1.95ab± 0.92	1.20a± 0.85	2.47b± 0.87
Penicillium sp.	1.22a± 0.66	1.07a± 0.94	1.37a± 0.74
Phomopsis sp.	0.24a± 0.48	0.27a± 0.53	0.27a± 0.54
Pythium sp.	0.85a± 0.48	0.80a± 0.85	0.69a± 0.65

Values in the same row and subtable not sharing the same subscript are significantly different at $p< .05$ in the two-sided test of equality for column means. Tests are adjusted for all pairwise comparisons using the Bonferroni correction.

Discussion

Soil is a most precious natural resource and contains the most diverse assemblages of living organisms. Indigenous microbial populations in soils are of fundamental importance for ecosystem functioning in both natural and managed agricultural soils (O'Donnell *et al*. 1994; Doran and Zeiss 2000). Microbial population size and community structure are sensitive to changes in chemical properties of the surrounding soil (Pansombat *et al.,* 1997; Tokuda and Hayatsu, 2002).

Fungal species typically have a wide pH optimum, often covering from 5–9 without significant inhibition of their growth (Wheeler *et al*., 1991; Nevarez *et al*., 2009). In this study, the pH range corresponds to the range observed to support active fungal growth and is most probably the reason for the rich fungal composition recorded in the sampled soils.

Soil organic matter levels in soils worldwide range from trace amounts up to 20%. There is currently no universally accepted soil organic matter threshold value for determining maximum agricultural productivity. Some soil may demonstrate maximum productivity at 1.2% organic matter content, the same soil organic matter value may indicate a degraded soil with limited soil productivity (Kay and Angers, 1999).

Microorganisms make up a small portion of the soil's organic matter (less than 5%). They are important to the formation, transformation, and functioning of the soil. In the soil, they conduct indispensable processes such as decomposition, nutrient cycling, and degradation of toxic materials, N fixation, symbiotic plant relationships, and pathogen control. Some fungi are known to parasitize insects while some are known to parasitize nematodes thereby keeping the numbers of these insects and nematodes in check.

Conclusion

Nine fungal species belonging to seven genera were isolated from the sampled agricultural soil. The distribution of these fungi within the sampled soil varied; their habitation in the soil is favored by the pH range and organic matter content levels of the soil.

Recommendation

Soils play key roles in agricultural sustainability, particularly, the production of adequate quantity and quality of the food which will promote human and animal health. As a major component of the soil microbial community, further studies into finding the best ways of maintaining fungal biodiversity with emphasis on their activities in the ecosystem including disease control and bioremediation are required.

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