

IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ON NUTRIENT UPTAKE AND THE GROWTH OF *C. RETUSA* AND *S. OCCIDENTALIS* UNDER PHOSPHORUS STRESS

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ABSTRACT

This study investigates the influence of Arbuscular Mycorrhizal Fungi (AMF) on the growth of *Crotalaria retusa* and *Senna occidentalis* under three phosphorus levels (low, medium, and high). Conducted in the experimental garden of Ahmadu Bello University Zaria, the soil samples were collected from a degraded site at the Institute of Agricultural Research, sieved, and sterilized. Perforated buckets were filled with sterilized soil, and the trench method was employed for AMF application. Three phosphorus levels were tested, and seeds of *C. retusa* and *S. occidentalis* were planted in individual buckets. Daily watering and observations were carried out for twelve weeks, measuring seedling height, leaf length, width, and number of leaves. The results indicate that high phosphorus concentration (12g/bucket) constrains the growth of *C. retusa*, while medium concentration (6g/bucket) enhances shoot length, branches, and leaves. AMF inoculation significantly improves growth attributes, but reduced growth in *C. retusa* under high phosphorus suggests potential incompatibility between phosphorus and AMF. At week 6, medium phosphorus (6g/bucket) resulted in more leaves (122.17 ± 37.61) than low and high levels. Lowest growth occurred at low phosphorus (0g/bucket). *Arbuscular mycorrhizal* fungi improved overall growth, but high phosphorus hindered *C. retusa* growth due to potential incompatibility with AMF. Overall, the study highlights the complex interplay between AMF, phosphorus levels, and plant growth, offering insights into optimizing conditions for the cultivation of *C. retusa* and *S. occidentalis*.

Keywords: Three Phosphorus levels, AM fungi, Nutrient acquisition, *Crotalaria retusa* and *Senna occidentalis*, Growth parameters.

INTRODUCTION

In arid and semi-arid regions of Nigeria, soil degradation is exacerbated by human factors such as massive deforestation, overgrazing, overcultivation, bush burning, and general land misuse. Vegetation plays a fundamental role in soil conservation (Monoz-Rojas *et al.*, 2016). In the tropics, soil erosion and depletion are becoming problems of global proportions, and few farming systems are immune to them. Integrating leguminous cover crops into the existing farming systems to address these problems has been very successful because of the high agronomic benefits of using these legumes (Loss *et al.*, 2001; Fosu *et al.*, 2004). Legumes and high nitrogen-fixing trees can be used in agricultural systems to replenish nitrogen, the most limiting growth factor in soils.

Phosphorus (P) is an essential macronutrient that constitutes about 0.2% of a plant's dry matter (Marschner, 1995). Phosphorus is required during the processes of energy generation and transfer, carbon metabolism, membrane synthesis, enzyme activation, and nitrogen fixation (Schachtman *et al.*, 1998) and is a constituent of key biomolecules like nucleic acids, phospholipids, and adenosine triphosphate (ATP) (Marschner, 1995). Limited P availability in soils is an important nutritional constraint to the growth of plants (Bates and Lynch, 2000). Phosphorus is the least mobile nutrient under most soil conditions, irrespective of total P contents in the soils (Hinsinger 2001; Schachtman *et al.*, 1998). Soils can be classified into two major groups with respect to total P contents: soils containing inherently low P contents, like acrisols or sandy soils, and other groups of soils that include nitisols, acid andosols, or calcareous and alkaline soils that contain a considerable amount of P, but whose major fraction is fixed with different soil constituents.

Application of P fertilizers is the most common practice to address the problem of low P availability in agricultural soils (Ramaekers *et al.*, 2010). However, this practice is confronted with the daunting challenges of the immobilization or precipitation of applied P with soil constituents, the depletion of nonrenewable P sources, and the high cost of P fertilizers (Vance *et al.*, 2003). Available P in most soils may constitute < 0.1% of total soil P (Khan *et al.*, 2009). In P-deficient soils, the use efficiency of applied P is very low, and >80% of applied P may be fixed on soil constituents or precipitated with Ca, Fe, and Al compounds and thus become unavailable to the plants (Gill *et al.*, 1994; Trolove *et al.*, 2003; Vance *et al.*, 2003) or converted to organic forms (Holford, 1997), and about 20% or less of P applied is removed by the crop in the first year after its application. According to the US geological survey, globally, 22 million metric tons of P are extracted from natural sources annually (Gaxiola *et al.*, 2011). Globally, P consumption is increasing by about 3% annually, and natural reserves may be depleted in the near future (Cordell *et al.*, 2009; Jasinski, 2008).

The nutrient-fixing abilities of legumes can be enhanced by *Rhizobium* spp. and by colonizing their roots by Arbuscular mycorrhiza fungi (Asim *et al.*, 1980). The arbuscular mycorrhiza fungi are present in Guinea Savannah soils (Auger, 2001; Smith and Read, 2008; Armugam *et al.*, 2010), but the full potential of applying them to management practices to improve crop yield is not achieved. AMF functions as conduits for the flow of energy and matter between plants and soil (Cardon and Whitbeck, 2007). AMF colonization improves the tolerance of plants to stressful cues by bringing about several changes in their morpho-physiological traits

(Algarawi *et al.*, 2014a; Hashem *et al.*, 2015). AMFs are considered natural growth regulators of most terrestrial flora and can also be bio-inoculants. Research encourages their use as prominent biofertilizers in sustainable crop productivity (Barrow, 2012).

It has also been reported that AMF-inoculant soils form more constant masses and significantly higher extraradical hyphal mycelium than non-AMF-treated soils (Syamsiyah *et al.*, 2018).

MATERIALS AND METHODS

The Study Area

The study was conducted in the Experimental Garden, Department of Botany, Ahmadu Bello University Zaria, on Lat. 11° N, Long 70 421E, and Altitude 660m. The area has a tropical climate, with the highest temperature in April and cold and dry Harmattan winds between November and January. This study was conducted between the 2018 and 2019 growing seasons.

Preparation of the Soil, Planting Buckets, Fertilizer, and AMF

Soil samples were collected from the degraded site of the Institute of Agricultural Research (IAR). These were sieved through a 6mm mesh and sterilized at 120 °C for two hours. Perforated buckets of 70kg were washed with tap water; these perforated buckets were filled with 7kg of sterilized, degraded soil.

The trench method of inoculums was used to apply the arbuscular mycorrhizal fungi. Five grams of the inoculums were used for thirty-six buckets (36) with their covers, which were kept for easier water drainage. The seeds of *C. retusa* and *S. occidentalis* were planted singly. The germination of *C. retusa* seeds started ten days after planting, while the seeds of *S. occidentalis* germinated two weeks after planting. Watering and observations were done regularly until germination was achieved.

Fertilizer Application

Phosphorus fertilizers were applied. The fertilizer was applied at three levels: low (0 g/bucket), medium (6 g/bucket), and high (12 g/bucket).

Data Collection

Data were collected at one-week intervals after planting for twelve (12) weeks on different plant stands. The seedlings were then measured from the root collar to the tip of the terminal shoot (using a meter rule) to determine the seedling height. Leaf length, leaf width, and leaf area were also measured and recorded.

RESULTS AND DISCUSSION

This investigation revealed a high significant difference ($p \leq 0.05$) in shoot lengths of *C. retusa* at weeks 1, 2, and 11 across the three applied phosphorus levels. However, the shoot length was higher at the medium level of phosphorus application (Table 1). It has also been noted that high phosphorus led to a high growth response in *C. retusa* inoculated with AMF, contrary to what was observed during higher levels of phosphorus application, where there was a decline in the growth of shoot length. This finding is in line with the work of Jha *et al.* (2012), who reported that high nutrients, especially phosphorus and water uptake by AM-inoculated plants, generally lead to secondary indirect effects such as improved plant growth (Shukla *et al.*, 2012). Lower growth of shoot length (28.90 ± 2.32 cm) at week 2 under high (12 g/bucket) levels of phosphorus compared to medium levels (35.82 ± 1.80 cm) could

result from incompatibility between phosphorus and the AMF. It has been reported that high soil nutrients, particularly phosphorus, correlate with low AMF colonization levels. A negative effect of the application of phosphorus on AMF colonization level, spore abundance, and the response of the plant to the application of AMF has been reported (Schweiger *et al.*, 1995; Dann *et al.*, 1996; Miller and Jackson, 1998).

Among the many inorganic nutrients plants require, phosphorus is an essential element that significantly affects plant growth and metabolism (Raghothama, 1999; Marschner, 2002). It is well documented that phosphorus is required for several physiological processes, including cell division, cell elongation, and bud growth (Marschner, 2002). Thus, based on the metabolic roles played by phosphorus, such an increase in plant growth weights could be expected as a result of phosphorus application. The pronounced effect of phosphorus on growth attributes has been recorded in many plants (Khan *et al.*, 2000; Samiullah and Khan, 2003; Naeem and Khan, 2005; Khan and Mohammad, 2006).

The growth of *S. occidentalis* increases with increased phosphorus concentration; the highest shoot length (34.50 ± 1.50 cm) at week 1 was observed at the high level of phosphorus application, which was followed by 34.50 ± 1.50 cm at the medium level and 18.47 ± 1.52 cm at the low phosphorus level. This effect could result from high phosphorus concentrations and plant-tissue interactions. It has been reported that, in the vegetative part of the plant, nutrients with impaired mobility in the phloem (such as Cu, Zn, Mn, and Ca) tend to increase during plant development. In contrast, a nutrient with very high mobility, such as phosphorus, tends to decrease in concentration, leading to decreased growth and development in plants (Wang *et al.*, 2020).

For the number of leaves, the medium level of phosphorus application has the highest number of leaves for the 12th week. At week one, the medium level was significantly higher than the low level. Also, there was no significant difference between medium and high at week one after planting. There was a significant difference between the three levels of phosphorus application two weeks after planting. In week 3, there was a significant difference between medium and low and medium and high, but there was no significant difference between low and high levels. There was no significant difference at all three levels for the number of leaves from weeks 4, 5, 6, 8, 9, 10, and 11 (Table 1).

The medium level has the highest leaf length from weeks 1–12 (Table 1). There was no significant difference between medium and low and medium and high P levels at weeks 2–9 and weeks 11 and 12, respectively, in *Crotalaria retusa* inoculated with AM fungi with different levels of phosphorus.

This investigation revealed that *C. retusa*'s decreased growth characteristics, as indicated in Table 1, may be the consequence of increased phosphorus application, which may have a negative impact on AMF function. In other words, limited biomass accumulation could result from high phosphorus input causing antagonism. Mycorrhizal associations tend to decrease when plant phosphorus concentration rises, which is a key obstacle to utilizing them in the agricultural system, according to research from earlier studies (Vaklentine *et al.*, 2001). According to Bruce *et al.* (1994), plant tissues that contain more phosphorus produce fewer spores

and secondary external hyphae.

Table 1: Growth response of *Crotalaria retusa* inoculated with *Arbuscular Mycorrhizal Fungi* with different levels of Phosphorous

Week	Dose	SL (cm)	NL	LL (cm)	NB	LW (cm)
1	High	22.05±1.61 ^a	21.67±2.26 ^a	5.08±0.18 ^a	1.33±0.21 ^b	2.02±0.10 ^a
	Medium	26.22±1.40 ^a	25.17±3.42 ^a	6.20±0.33 ^a	2.80±0.58 ^a	2.25±0.07 ^a
	Low	12.17±2.09 ^b	9.67±1.15 ^b	3.35±0.61 ^b	1.00±0.00 ^b	1.22±0.29 ^b
2	High	28.90±2.32 ^b	30.17±4.39 ^b	5.87±0.36 ^a	2.83±0.60 ^a	2.11±0.10 ^a
	Medium	35.82±1.80 ^a	46.17±4.92 ^a	5.82±0.33 ^a	4.33±0.49 ^a	2.22±0.14 ^a
	Low	19.72±1.63 ^c	15.67±1.45 ^c	5.02±0.32 ^a	0.67±0.42 ^b	1.88±0.15 ^a
3	High	31.08±2.59 ^{ab}	37.67±5.29 ^b	5.72±0.49 ^a	4.00±0.63 ^a	2.20±0.12 ^a
	Medium	35.33±5.57 ^a	63.33±12.71 ^a	6.32±0.80 ^a	4.80±0.37 ^a	2.33±0.32 ^a
	Low	23.00±1.57 ^b	25.50±2.22 ^b	5.62±0.21 ^a	3.00±0.71 ^a	2.03±0.14 ^a
4	High	37.30±3.12 ^a	45.50±7.46 ^a	5.98±0.47 ^a	4.17±0.79 ^{ab}	6.25±3.95 ^a
	Medium	41.83±6.54 ^a	68.42±20.24 ^a	7.10±0.95 ^a	6.80±1.24 ^a	2.37±0.33 ^a
	Low	31.47±2.33 ^a	34.83±4.34 ^a	6.93±0.57 ^a	3.33±0.76 ^b	2.57±0.20 ^a
5	High	41.92±3.42 ^a	59.50±11.55 ^a	5.88±0.34 ^a	5.33±.84 ^a	2.52±0.20 ^a
	Medium	44.87±9.58 ^a	90.67±26.11 ^a	6.07±0.47 ^a	6.40±1.86 ^a	2.55±0.20 ^a
	Low	38.18±3.27 ^a	53.33±8.74 ^a	6.88±0.55 ^a	4.00±0.58 ^a	2.50±0.15 ^a
6	High	50.13±3.34 ^a	70.17±15.31 ^a	5.73±0.50 ^a	6.00±1.10 ^b	2.35±0.21 ^a
	Medium	53.17±10.67 ^a	122.17±37.61 ^a	7.58±0.88 ^a	9.75±1.25 ^a	2.53±0.32 ^a
	Low	44.53±4.04 ^a	67.83±10.77 ^a	6.67±0.73 ^a	4.50±0.72 ^b	2.72±0.19 ^a
7	High	54.53±3.96 ^a	85.17±25.31	5.32±0.57 ^a	7.33±2.20 ^b	2.40±0.21 ^a
	Medium	60.57±10.53 ^a	133.83±39.59	6.73±0.62 ^a	14.25±1.49 ^a	2.83±0.13 ^a
	Low	51.03±3.57 ^a	80.33±12.42	6.17±0.41 ^a	4.83±0.54 ^b	2.42±0.18 ^a
8	High	59.77±3.38 ^a	103.33±38.52 ^a	5.88±0.58 ^a	9.50±3.95 ^{ab}	2.45±0.22 ^a
	Medium	66.18±9.96 ^a	146.33±42.10 ^a	7.83±0.63 ^a	18.75±2.69 ^a	3.03±0.15 ^a
	Low	48.52±8.82 ^a	87.67±22.43 ^a	6.35±0.91 ^a	5.20±0.58 ^b	2.48±0.30 ^a
9	High	65.33±3.63 ^a	109.50±38.58 ^a	6.05±0.56 ^a	9.67±4.11 ^a	2.72±0.21 ^a
	Medium	69.00±9.61 ^a	160.33±45.45 ^a	7.70±0.51 ^a	13.83±4.65 ^a	3.08±0.13 ^a
	Low	51.72±8.69 ^a	96.67±23.11 ^a	6.63±0.82 ^a	4.83±1.17 ^a	2.62±0.28 ^a
10	High	72.92±3.90 ^a	63.88±13.08 ^b	5.83±0.50 ^b	13.17±5.82 ^{ab}	2.68±0.21 ^b
	Medium	81.47±7.94 ^a	167.33±45.60 ^a	9.00±0.52 ^a	25.25±2.18 ^a	3.48±0.16 ^a
	Low	57.02±10.13 ^a	103.83±28.39 ^{ab}	6.93±0.92 ^b	5.40±0.51 ^b	2.87±0.34 ^{ab}
11	High	77.83±3.05 ^{ab}	126.33±41.08 ^a	5.83±0.46 ^b	16.83±5.75 ^a	2.75±0.15 ^a
	Medium	92.50±4.07 ^a	176.50±44.15 ^a	8.63±0.62 ^a	19.83±5.50 ^a	3.45±0.27 ^a
	Low	63.55±10.04 ^b	110.17±28.28 ^a	7.20±0.95 ^{ab}	6.20±0.58 ^a	3.15±0.28 ^a
12	High	96.02±4.70 ^a	149.17±45.40 ^a	6.03±0.41 ^b	20.83±6.74 ^a	2.60±0.26 ^a
	Medium	102.50±4.46 ^a	199.50±45.01 ^a	9.35±0.55 ^a	26.67±6.85 ^a	3.40±0.38 ^a
	Low	82.78±12.67 ^a	125.33±31.28 ^a	7.15±0.79 ^b	11.00±2.88 ^a	2.92±0.23 ^a
		0.256	0.447	0.005	0.235	0.197

Legend: SL = Shoot length, NL = number of leaves, LL = Leaf length, NB = number of branches, LW = Leaf width. Low = 0g, Medium = 6g and High = 12g

**Means sharing the same superscript (down the column) are not significantly different from each other ($p \geq 0.05$)

The growth attributes of *S. occidentalis* inoculated with AMF were observed to increase with increased P levels (Table 2). The highest shoot length (SL) after the 6th week of planting was observed to be 48.13±3.91 cm. This was followed by medium and low levels of P application with average mean values of 39.92±2.84 and 29.00±5.00, respectively (Table 2). Similarly, the number of leaves (NL) was found to increase with increased phosphorus levels at

week five (5), with low phosphorus application having the lowest number of leaves, followed by medium and high P with average mean values of 39.67±3.84, 45.83±2.65, and 52.50±0.15, respectively, as shown in Table 2. Although the increase in SL was observed to correspond with increased phosphorus application, as shown in Table 2, This increase was not statistically significant at weeks 8 and 9 (Table 2). This finding is in line with the work of Turk

et al. (2003), who reported that phosphorus fertilization is vital for root, shoot, and flower development, energy translocation, and other metabolic processes in plants. It is also highlighted that an optimum phosphorus supply at the early stage of plant growth and cell elongation can lead to complete development and increased growth attributes (Spencer and Chan, 1991; Turk et al., 2003). This rise in growth is in line with recent research by Klinsukon et al. (2021) that discovered that AMF-inoculated crops showed increased photosynthetic efficacy in challenging environmental circumstances. Talaat and Shaky (2014) have reported that AMF symbiosis enhances photosynthetic rate, stomatal conductance, and leaf water relations in salinized settings.

Table 2: Growth response of *Senna occidentalis* inoculated with Arbuscular Mycorrhizal Fungi with different levels of phosphorous

Week	Dose	SL	NL	LL	NB	LW
1	High	34.50±1.50 ^a	37.50±0.50 ^a	5.95±0.45 ^a	3.25±0.15 ^a	
	Medium	29.83±1.34 ^a	31.67±2.50 ^a	4.45±0.40 ^{ab}	2.67±0.16 ^b	
	Low	18.47±1.52 ^b	28.00±2.52 ^a	3.00±0.38 ^b	2.00±0.17 ^b	
		0.001	0.209	0.017	0.013	
2	High	40.50±0.50 ^a	47.00±5.00 ^a	5.60±0.60 ^a	3.40±0.20 ^a	
	Medium	32.50±0.85 ^b	33.83±3.30 ^{ab}	5.20±0.50 ^a	2.67±0.38 ^a	
	Low	21.33±0.88 ^c	31.00±4.04 ^b	4.80±0.31 ^a	2.43±0.18 ^a	
		0.000	0.114	0.708	0.394	
3	High	47.00±1.00 ^a	45.50±0.50 ^a	5.25±0.25 ^a	3.30±0.20 ^a	
	Medium	34.07±0.91 ^b	37.33±3.07 ^{ab}	5.38±0.49 ^a	2.58±0.31 ^a	
	Low	24.67±1.45 ^c	31.33±1.45 ^b	5.27±0.41 ^a	2.43±0.12 ^a	
		0.000	0.093	0.980	0.307	
4	High	49.50±0.50 ^a	49.50±2.50 ^a	5.55±0.45 ^a	3.10±0.50 ^a	
	Medium	37.52±1.15 ^b	42.00±2.15 ^a	6.32±0.43 ^a	2.78±0.26 ^a	
	Low	27.33±1.33 ^c	44.33±8.84 ^a	5.83±0.33 ^a	2.700.12 ^a	
		0.000	0.598	0.549	0.734	
5	High	52.50±1.50 ^a	52.50±0.15 ^a	6.75±0.15 ^a	2.70±0.20 ^a	
	Medium	39.78±1.28 ^b	45.83±2.65 ^{ab}	6.07±0.33 ^a	2.78±0.09 ^a	
	Low	30.23±1.53 ^c	39.67±3.84 ^b	5.53±0.26 ^a	2.40±0.17 ^a	
		0.000	0.129	0.212	0.149	
6	High	48.13±3.91 ^a	55.75±4.07 ^a	6.70±0.24 ^a	2.98±0.06 ^a	
	Medium	39.92±2.84 ^{ab}	44.33±3.33 ^{ab}	6.30±0.39 ^a	2.95±0.13 ^a	
	Low	29.00±5.00 ^b	36.00±15.00 ^b	5.75±0.55 ^a	2.35±0.05 ^b	
		0.040	0.120	0.429	0.030	
7	High	49.63±3.85 ^a	58.00±3.67 ^a	6.25±0.20 ^a	2.93±0.08 ^a	
	Medium	41.07±3.11 ^{ab}	48.67±3.38 ^a	6.10±0.29 ^a	2.77±0.20 ^a	
	Low	30.00±6.00 ^b	38.50±17.50 ^a	5.90±0.40 ^a	2.40±0.00 ^a	
		0.047	0.174	0.798	0.321	
8	High	51.75±3.65 ^a	60.50±0.16 ^a	6.95±0.16 ^a	2.95±0.10 ^a	
	Medium	35.47±6.82 ^a	53.50±3.55 ^a	6.73±0.40 ^a	2.87±0.20 ^a	
	Low	30.75±6.75 ^a	40.00±19.00 ^a	6.45±0.15 ^a	2.50±0.10 ^a	
		0.154	0.196	0.750	0.408	
9	High	42.68±13.01 ^a	61.75±4.50 ^a	6.75±0.30 ^a	3.00±0.15 ^a	
	Medium	46.65±3.91 ^a	54.67±4.79 ^a	6.92±0.35 ^a	2.95±0.20 ^a	
	Low	32.00±8.00 ^a	40.50±19.50 ^a	6.20±0.10 ^a	2.65±0.25 ^a	
		0.593	0.254	0.509	0.621	
10	High	55.83±3.58 ^a	67.75±6.50 ^a	6.73±0.18 ^a	3.05±0.07 ^a	
	Medium	49.03±3.86 ^a	58.67±4.49 ^{ab}	6.67±0.33 ^a	2.73±0.18 ^{ab}	
	Low	32.50±8.50 ^b	38.50±17.50 ^b	6.20±0.10 ^a	2.35±0.05 ^b	
		0.047	0.101	0.627	0.096	
11	High	62.83±3.68 ^a	73.75±5.95 ^a	6.68±0.60 ^a	2.60±0.07 ^a	
	Medium	55.23±4.16 ^a	63.50±6.10 ^a	5.85±0.58 ^a	2.38±0.09 ^a	
	Low	34.50±10.50 ^b	36.00±15.00 ^b	6.35±0.05 ^a	2.55±0.15 ^a	
		0.029	0.048	0.606	0.243	
12	High	66.40±5.06 ^a	81.25±7.50 ^a	6.68±0.32 ^a	2.60±0.11 ^a	
	Medium	58.00±3.74 ^a	61.17±9.11 ^{ab}	6.17±0.41 ^a	2.45±0.13 ^a	
	Low	36.00±12.00 ^b	37.00±16.00 ^b	5.75±0.55 ^a	2.25±0.15 ^a	
		0.020	0.084	0.466	0.382	
	P-value	0.020	0.084	0.466	0.382	

Legend: SL = Shoot length, NL = number of leaves, LL = Leaf length, NB = number of branches, LW = Leaf width. Low = 0g, Medium = 6g and High = 12g

**Means sharing the same superscript (down the column) are not

significantly different from each other ($p \geq 0.05$)

Conclusion

In conclusion, this investigation clearly revealed that shoot length (SL), number of leaves (NL), and number of branches (NB) of *S. occidentalis* inoculated with AM fungi linearly increased with increasing concentrations of P during vegetative growth, while there was gradually decreased growth at high (12 g/bucket) phosphorus application under the same condition for *C. retusa*. As reported in the earlier findings, phosphorus supply is critical for many field-grown crops during the early stages of growth. However, the reduced growth attributes of *C. retusa* under AMF-induced conditions during high phosphorus levels result in antagonism between P and AMF, leading to reduced growth.

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