

EFFECT OF *PYTHIUM APHANIDERMATUM* ON TWO CULTIVARS OF PEPPER (*Capsicum* spp)

Aliyu, T. H., Balogun, O. S., and Adesina, O. M.

ABSTRACT

Department of Crop protection, Faculty of Agriculture, University of Ilorin, P.M.B 1515 Ilorin- Nigeria.
Email of Corresponding Author: aliyutaiyehussein@yahoo.com, Telephone: +2348030472667

A potted experiment was carried out to study the effect of *Pythium aphanidermatum* on two cultivars of pepper [NHVI^B (improved variety) and Sombo (local variety)]. 24 pots of sterilized soil were used and four seeds of each pepper variety were planted in 12 pots each. The plants were inoculated by drenching at planting (pre-emergence) and 2 weeks later (post-emergence) and non-inoculated plants served as control. The inoculum was pure isolates of *P. aphanidermatum*, obtained from sub-culturing colonies of the fungus that was growing on infected okra root tissues. Data were collected for growth and yield indices. The data were subjected to analysis of variance (ANOVA) and significant differences determined at $P \leq 0.05$. The results showed that there were significant differences in the growth and yield parameters between the inoculated and the control plants, with the inoculated plants having lower values. Pre-emergence inoculation appeared to be more effective than post-emergence inoculation in inducing disease response in plants. The improved variety NHVI^B generally performed better than the local variety. The results suggest that prevention of early fungi infection and the availability of disease resistant cultivars could be the best control strategies in reducing the diseases caused by *Pythium* spp on pepper.

Keywords: *Pythium aphanidermatum*, Pepper cultivar, Pre-emergence, Post-emergence, Inoculation.

INTRODUCTION

Peppers are vegetable crops belonging to the family Solanaceae and genus *Capsicum*, which are native to tropical America and Africa. The family consists of about 90 genera and nearly 3000 species (Vidarth and Tripatha 2002). Chillis and other species come from the bushy *Capsicum* species and white or black pepper come from climbing vine, *Piper nigrum* (Vaughan and Geissler, 2009). It is generally believed that about 20 *Capsicum* species are distributed worldwide out of which *Capsicum annum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum pendulum* and *Capsicum pubescens* are the five major species cultivated (Saikat and Amit, 2003). *Capsicum* species is among the world's most popular vegetable and is being used mainly as spices and condiments. It is an important spice crop that is produced and consumed either fresh or processed (Sonago, 2003). Some varieties are very hot (the chilli and cayenne pepper) and are normally used as a pungent flavouring whilst milder varieties (the sweet pepper) have a very pleasant flavour with a slight sweetness and are often eaten raw in salads (Brouk, 1975). *Capsicum frutescens* contain about 85.7% moisture, 4.35% ash, 11.7% crude protein, 12.7% crude fat, 9 – 17% crude fibre and 62.94% carbohydrate. They are rich in mineral elements such as Sodium, Calcium, Iron, Phosphorus, Potassium, Zinc, Copper, Cobalt, Manganese, Sulphur and Magnesium (Mehnet et al., 2006) and also have good quantities of vitamins E, B₁, B₂, B₃, B₅, B₆, C Carotene and folate (USDA, 2008). Pepper is an important vegetable consumed by human beings in Nigeria and is mainly grown around the savannah ecological zones as a crop mixture or mono crop both during the rainy season and dry season under irrigation (Denton et al., 2000). It is also used in fresh form or as paste, pure and juice or ground with other vegetables in the preparation of stew and soup (NAERLS).

Diseases are major constraints to pepper production in sub-Saharan Africa (Nsabiyera et al., 2012). The major limiting diseases of most peppers in Sub-Sahara Africa and elsewhere are phyto-pathogenic fungi, bacteria, and viruses (Melanie and Sally, 2004). Attacks by fungal, bacterial or viral diseases, nematodes, mites and many insect pest infestations infections can cause significant losses in pepper production (Ochoa-Alejo and Ramirez-Malagon, 2001). *Pythium* is one of the most important root and seedling pathogens in greenhouses, on both vegetables and horticultural crops (Paulitz and Belanger 2001). *Pythium aphanidermatum* is a soil borne plant pathogen in a genus in the class *Oomycetes*, which are also known as water moulds (Sutton et al., 2006). Root browning is a principle symptom of *Pythium* root rot, which is a destructive disease of peppers (Moorman 2002; Owen-Going et al., 2003). The root browning, marks the development of the necrotrophic phase of the disease, which is associated with abrupt decline in the growth rate of the roots and foliage of affected pepper plants (Sutton et al., 2006). The disease development of *P. aphanidermatum* is still rather unpredictable (Postma et al., 2001). Rapid germination of sporangia of *Pythium* after exposure to exudates or volatiles from seeds or roots (Osburn et al., 1989) followed by immediate infection makes management of the pathogen very difficult (Whipps and Lumsden, 1991). Although fungicides have shown promising results in controlling the damping-off disease, phytotoxicity and fungicide residues are major problems leading to environmental pollution and human health

hazards (Ramamoorthy *et al.*, 2002). Development of fungicide resistance by *Pythium* spp. further discourages its use for disease control (Punja and Yip 2003). Pests and diseases are best managed using host resistance, which is a cheap option for farmers (Duveiller *et al.*, 2007).

The implication of making available good cultivars to the farmers and development of such cultivars within a short period of time are obvious factors to sustainable agricultural productivity in developing countries. The availability of disease-resistant cultivars would therefore be the best control strategy in reducing the diseases caused by *Pythium* spp on pepper. The objectives of the present study were to determine the effect of inoculation of *Pythium aphanidermatum* and influence of varietal differences on the response of the pepper cultivars.

MATERIALS AND METHODS

Source of seeds: The two pepper varieties used for the experiment, were NHVI^B and SOMBO (local), which were respectively obtained from the Nigerian Horticultural Research Institute (NIHORT) Ibadan – Oyo State and the Ipata market in Ilorin – Kwara State. **Source of inoculum, preparation of medium and plating:** Infected okra plants showing symptoms of root-rot were collected from vegetable gardens around River Oyun in Ilorin-Nigeria. Soil samples (500 g each) were also collected from the rhizosphere of the infected roots of okra and the samples transported to the laboratory almost immediately. The roots of the diseased okra plants were then chopped into small pieces and their respective root soil washed into separate petri dishes. 3.9g of potato-dextrose-agar (PDA), was added to 100ml of distilled water in 500ml conical flask which had been thoroughly sterilized. The conical flask containing the PDA, was then covered with cotton wool and aluminium foil and further sterilized in an autoclave to a temperature of 120°C. The conical flask was then allowed to cool to 45°C and 1ml of streptomycin was then added to suppress bacterial growth. The medium was then aseptically poured into sterile plates and left to solidify (APHA, 1992). The chopped roots were then inoculated at 3 different points in 4 agar plates. The serial dilution method according to Amoo *et al.* (2007), was used to isolate pathogens from the contaminated soil. The isolated fungal pathogens were then inoculated at 3 different points inside 4 other plates. All 8 plates were then incubated at 25°C for 3 days. The pure cultures of the fungal pathogen were obtained by sub-culturing the colonies growing from the infected root tissues and soils. Colony observations were made under a Leica microscope and fungi identification was done using laboratory manuals (Samson and Pitt, 2000).

Preparation of inoculum: 3 petri-dishes containing pure isolates (fungal isolator) were macerated in 500ml of distilled water in a waring blender. 10ml of the concentrated inoculum (fungal isolate), was then suspended in 100ml of water, and this served as the fungal inoculum.

Experimental design: The experiment was designed as a factorial fitted in a completely randomized design. 24 plastic pots (15cm- long), were filled with sandy-loam soil autoclaved at 180°C for 8hours, to eliminate soil inhabiting pathogenic micro-organisms (Fernando and Linderman, 1993). The sterile soil was left for three days to cool before packing into the sterile pots. The potted soil was then watered and left for 72hours before sowing. Four seeds of each variety were planted in each pot and each treatment, including the non-inoculated pots that served as the experimental control were replicated thrice. Pre and post emergence inoculation were by drenching at sowing and at 14days after planting respectively and this was done according to the method of Agrios (2005). The experimental pots were placed in the pavilion (27 – 30°C) under natural daylight conditions and the plants were watered daily.

Data collection and analysis: Data were collected in the course of the experiment for parameters such as: plant height, number of leaves per plant, number of flowers per plant, number of branches per plant, number of fruits per plant, fresh and dry root weight per plant, fresh and dry shoot weight per plant, fresh and dry fruit weight per plant and total number of fruits per plant. Data were subjected to analysis of variance (ANOVA) and the significant differences between them were determined at $P \leq 0.05$ using the New Duncan Multiple Range Test.

RESULTS

Identification of fungal pathogen: Observations carried out with the aid of Leica microscope on fungi isolated from the roots of infected okra plants, indicated that the pathogen possessed the following morphological characteristics: A fast growing fungus, with a white thallus with a hyaline, nonseptate, mycelium and the oospore does not fill the oogonium. Similar features have been recorded by Vawdrey and Westerhuis (2007), Suleiman (2010) and the pathogenic agent identified as *Pythium aphanidermatum*. **Effect on plant growth:** The effects of variety and inoculation on plant height is shown in Table 1. The pepper variety NHVI^B had the significantly taller plants compared to the local pepper variety from 4th to the 11th week after germination. At 49 days after germination, the local variety had an average plant height of 9.8cm and variety NHVI^B had an average plant height of 15.9cm. But at the 12th and 13th week after germination the results showed that varietal effect on plant height, was of no significant effect. Inoculation effect showed that the inoculation caused a reduction in plants

height, as the non-inoculated plants were apparently taller and there was significant differences in the heights from the 8th to the 14th week after germination. At 91 days after germination, the non-inoculated plants had the significantly taller plants (31.7cm). The time of inoculation also had significant effect on plant height. At the 4th, 5th, 8th, 9th, 11th and 12th weeks after germination, inoculation post-emergence produced the taller plants (5.5cm, 9.0cm, 15.6cm, 18.1cm, 20.5cm and 25.0 cm respectively).

Effect on number of leaves: Table 2 shows the effect of treatments on number of leaves per plant. The table shows that variety NHVI^B had a significantly higher number of leaves per plant from the 28th to the 63rd day after germination, with an average of 23.5 leaves per plant at the 9th week after germination. However thereon to the 91st day after germination, there were no significant differences between the two varieties. Inoculation effects were significantly different from the 35th to the 91th day after inoculation. The table shows that the non-inoculated plants had significantly higher number of leaves compared with the inoculated plants. At the 35th day after germination, the non-inoculated plants had an average of 10.6 leaves per plant while the inoculated plants had an average of 9.2 leaves per plant. By the 91st day after germination, the non-inoculated plants averaged 43.3 leaves per plant. The effect of time of inoculation was significant at the 35th, 56th, and 77th days after germination and for these days, plant inoculated post-emergence had the significantly higher number of leaves per plant of 10.8, 18.2 and 29.3 respectively.

Effect on number of flowers: The effect of the treatments on the number of flowers per plant is shown in Table 3. The effect of variety on the average number of flowers per plant, shows that the local variety were significantly higher at the 63rd DAP (8.2), 70th DAP (9.4), 77th DAP (8.6), and 84th DAP (8.2). The non-inoculated plants had the significantly higher number of flowers except at the 63rd DAP where the number of flowers was not significantly different between treatments. Time of inoculation had no significant effect on number of flowers per plant. Effect on number of fruits: Table 4 shows the effect of treatments on the number of fruits per plant. Varietal difference was of no significant effect on the number of fruits per plant. However, the non-inoculated plants had the significantly higher number of fruits per plant from the 63rd DAG (0.6) to the 91st DAG (1.8). Inoculation at pre-emergence were significantly higher at the 77th DAP (1.1), 84th DAG (1.3) and 91st DAG (1.3).

Effect on yield components: The effect of variety and inoculation on some biological and yield components is shown in Table 6. The table shows that variety NHVI^B had the higher yield parameters for dry shoot weight per plant (1.6g), fresh fruit weight per plant (4.0g), and dry fruit weight per plant (1.0g). The inoculation effect shows that the non-inoculated plants had the significantly higher yields for all the components, while time of inoculation effect was only significantly different for the dry root weight per plant, as the plants inoculated post-emergence had the significantly higher value of 1.0g, while the pre-emergence plants had a lower value of 0.7g.

Table 1: Effect of variety and inoculation on plant height (cm) at different days after germination

	Days after germination									
	28	35	42	49	56	63	70	77	84	91
Variety										
Local	3.7b	5.9b	7.8b	9.8b	12.3b	14.8b	17.4b	20.5b	23.6	25.6
NHVI ^B	6.3a	10.7a	13.2a	15.9a	17.5a	19.2a	21.2a	22.7a	24.5	26.8
S.E	0.288	0.414	0.444	0.487	0.422	0.435	0.522	0.434	0.521ns	0.495ns
Inoculation										
Inoculated	4.8	8.1	10.1	12.2b	14.1b	15.6b	17.2b	18.7b	19.8b	20.6b
Non-inoculated	5.2	8.5	10.9	13.5a	15.7a	18.4a	21.5a	24.5a	28.3a	31.7a
S.E	0.258ns	0.414ns	0.444ns	0.487	0.422	0.435	0.522	0.434	0.521	0.495
Time of Inoculation										
Pre-emergence	4.5b	7.7b	10.0	12.4	14.2b	16.0b	20.6a	22.8a	23.1b	27.0a
Post-emergence	5.5a	9.0a	10.9	13.2	15.6a	18.1a	18.1b	20.5a	25.0a	25.3b
S.E	0.288	0.414	0.444ns	0.487ns	0.422	0.435	0.522	0.434	0.521	0.495

ns = Not significant

Means with the same letter (s) within each column are not significantly different at $P \geq 0.05$ using the New Duncan's Multiple Range Test.

Discussion: *Pythium aphanidermatum* is a principal pathogen contributing to root rot of pepper and other hydroponic greenhouse crops (Zeng et al., 2000). It is difficult to accurately detect the onset or monitor the progression of root diseases such as *Pythium* root rot in commercial environments, especially in crops with high plant densities, dense canopies, and reduced accessibility of roots to direct observation. Disease can go undetected when all plants in a cohort are similarly stunted and not exhibiting wilting, chlorosis, or noticeable defoliation (Owen-Going, 2002). The results of this experiment shows that *Pythium aphanidermatum* causes growth and

Table 2: Effect of variety and inoculation on number of leaves per plant at different days after germination

	Days after germination									
	28	35	42	49	56	63	70	77	84	91
Variety										
Local	4.0b	7.5b	10.1b	12.1b	15.3b	20.5b	26.4	28.7	27.8	28.6
NHVT ^B	7.0a	12.3a	16.3a	19.5a	19.5a	23.5a	25.8	28.2	29.2	29.8
S.E	0.279	0.415	0.475	0.518	0.560	0.569	0.532ns	0.402ns	0.736ns	1.111ns
Inoculation										
Inoculated	5.7	9.2b	12.4b	14.6b	16.7b	20.8b	21.5b	26.5b	28.6b	29.2b
Non-inoculated	6.0	10.6a	14.0a	17.0a	18.2a	23.2a	30.8a	36.3a	43.3a	49.3a
S.E	0.279ns	0.415	0.475	0.518	0.566	0.569	0.532	0.402	0.736	1.111
Time of Inoculation										
Pre-emergence	5.5	9.0b	12.7	15.5	16.7b	22.0	26.3	27.5b	28.0	29.0
Post-emergence	6.2	10.8a	13.8	16.1	18.2a	22.0	25.9	29.3a	29.0	29.4
S.E	0.279ns	0.415	0.475ns	0.578ns	0.566	0.569ns	0.532ns	0.402	0.736ns	1.111ns

ns = Not significant

Means with the same letter (s) within each column are not significantly different at $P \geq 0.05$ using the New Duncan's Multiple Range Test.

Table 3: Effect of variety and inoculation on number of flowers per plant at different days after germination

	Days after germination					
	56	63	70	77	84	91
Variety						
Local	2.6	8.2a	9.4a	8.6a	8.2a	7.8
NHVT ^B	2.7	3.2b	5.1b	5.6b	5.4b	6.8
S.E	0.184ns	0.212	0.193	0.285	0.395	0.564ns
Inoculation						
Inoculated	1.7b	5.5	7.0b	5.3b	3.3b	2.1b
Non-inoculated	3.6a	5.8	7.5a	9.1a	10.3a	12.5a
S.E	0.184	0.212ns	0.193	0.285	0.395	0.564
Time of Inoculation						
Pre-emergence	2.5	5.6	7.4	7.4	6.5	7.5
Post-emergence	2.8	5.8	7.1	7.0	7.1	7.1
S.E	0.184ns	0.212ns	0.193ns	0.285ns	0.395ns	0.564ns

ns = Not significant

Means with the same letter (s) within each column are not significantly different at $P \geq 0.05$ using the New Duncan's Multiple Range Test.

Table 4: Effect of variety and inoculation on number of fruits per plant at different days after germination

	Days after germination				
	63	70	77	84	91
Variety					
Local	0.4	0.5	0.8	1.0	1.0
NHVT ^B	0.3	0.5	1.0	1.3	1.3
S.E	0.135ns	0.185ns	0.168ns	0.156ns	0.156ns
Inoculation					
Inoculated	0.2b	0.3b	0.3b	0.3b	0.3b
Non-inoculated	0.6a	0.8a	1.3a	1.8a	1.8a
S.E	0.135	0.185	0.68	0.156	0.156
Time of Inoculation					
Pre-emergence	0.3	0.6	1.1a	1.3a	1.3a
Post-emergence	0.5	0.4	0.6b	1.0b	1.0b
S.E	0.135ns	0.185ns	0.168	0.156	0.156

ns = Not significant,

Means with the same letter (s) within each column are not significantly different at $P \geq 0.05$ using the New Duncan's Multiple Range Test.

yield reductions in pepper varieties as a result of root browning. Bugbee and Monje (1992); Owen-Going *et al.* (2003), had in similar experiments reported that the expansive root browning caused by *P. Aphanidermatum*, was responsible for the substantial growth and yield reductions observed. in peppers grown in sterilized soil.

Table 5: Effect of variety and inoculation on some biological and yield components

	Fresh root wt Plant ⁻¹ (g)	Fresh shoot wt plant ⁻¹ (g)	Dry root wt plant ⁻¹ (g)	Dry shoot wt plant ⁻¹ (g)	Fresh fruit wt plant ⁻¹ (g)	Dry fruit wt plant ⁻¹ (g)	Total no. of fruit plant ⁻¹
Variety							
Local	4.8	6.3	0.9	1.5b	2.6b	0.7b	3.5
NHVI ^B	5.4	6.8	0.8	1.6a	4.0a	1.0a	4.3
S.E	0.357ns	0.421ns	0.067ns	0.052	0.523	0.133	0.665ns
Inoculation							
Inoculated	2.5b	3.5b	0.5b	0.8b	1.4b	0.3b	1.4b
Non-inoculated	7.7a	9.6a	1.2a	2.3a	5.2a	1.3a	6.3a
S.E	0.357	0.421	0.067	0.052	0.523	0.133	0.665
Time of Inoculation							
Pre-emergence	4.7	6.3	0.7b	1.5	3.9	1.0	4.4
Post-emergence	5.6	6.8	1.0a	1.6	2.8	0.7	3.3
S.E	0.357ns	0.421ns	0.067	0.052ns	0.523ns	0.133ns	0.665ns

ns = Not significant

Means with the same letter (s) within each column are not significantly different at $P \geq 0.05$ using the New Duncan's Multiple Range Test.

These reductions according to Woodrow *et al.*, (1988), is due to wilting and water stress which was accentuated by the fungal disease development in the roots. This phenomenon caused a reduction in the availability of photo assimilates for leaf growth in the inoculated pepper plants. The observed differences in the reaction of the local and NHVI^B pepper varieties to inoculation with *P. aphanidermatum*, could be due to different resistant capabilities in the two pepper varieties. This is also in agreement with Wutscher (1998) that resistance between varieties of a single plant species, often resides in physiological or biochemical differences between the resistant and susceptible varieties. The experiment also showed that inoculation at pre-emergence was generally more pathogenic and resulted in greater growth and yield reductions. This finding is in agreement with Agrios (2005), who stated that young plants are more susceptible to diseases compared to older plants. It is generally accepted that a plants susceptibility or resistance to disease depends largely on the age of the host plant at the time of arrival of the pathogen. This is because as a plant gets older, the tissues become more lignified therefore making it difficult for pathogen to penetrate into the tissues.

CONCLUSION AND RECOMMENDATION

The various damages caused by *Pythium spp* on pepper varieties are seriously putting the farmers at a loss. However, control using chemicals is a remote possibility for the majority of the farmers due to accessibility to these inputs and availability of resources to purchase them. This work concludes that inoculation of pepper varieties at pre-emergence was more pathogenic to the plant. The local pepper variety also appeared to be more susceptible to infection than the improved variety which exhibited a higher level of tolerance. It is hereby recommended that the cultivation of improved pepper varieties, such as variety NHVI^B be encouraged and fungal infection at the early stages of plant emergence, should be avoided.

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