# EFFECT OF SOME PATHOGENIC MICROORGANISMS ON GERMINATION AND SEEDLING GROWTH OF *HIBISCUS SABDARIFFA*

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## ABSTRACT

A study was carried out to find out the effect of pathogenic bacteria and fungi on seed germination and seedling growth of *Hibiscus sabdariffa*. The fungi utilized are *Aspergillus flavus* Link Ex fr, *Aspergillus niger* Van Tieghem, *Fusarium oxysporum* Schlecht, *Penicillium chrysogenum* Thom and *Penicillium roqueforti* Thom and the bacteria are *Arthrobacter* sp, *Erwinia* sp, *Lactobacillus* sp and *Corynobacteria* sp. Effects of the individual micro-organisms on seed germination and seedling growth showed diverse degree of inhibition on the growth parameters. *Erwinia sp.* had the least reduction in germination (20%) when compared with the control plant which showed 100% yield. *Lactobacillus sp.* and *Corynebacterium sp*. had the next reduction (20.4%), *Arthrobacter sp.* (25%), *F. oxysporum* (20.8%), *A. flavus* (30.0%) *P. chrysogenum* and *A. niger* (30.2%) *P.roqueforti* (40%). The study showed that the fungus *Fusarium oxysporum* and the bacteria *Erwinia* sp. negatively affected seed germination and seedling growth of *H. sabdariffa.* They were the most pathogenic among the tested microorganisms.

**Keywords:** *Hibiscus sabdariffa*, pathogenic microorganisms, germination,seedling growth

## INTRODUCTION

*Hibiscus sabdariffa* Linn (Roselle) is an angiosperm of the family Malvaceae. It is an annual herb that grows to 180 cm or more; stems are glabrous, the lower leaves are ovate with the upper leaves being 3–5 palmately lobed. Roselle seeds germinate within 2 to 3 days. Seedlings may be raised in nursery beds and transplanted when 7.5-10 cm high. The seeds are usually set directly in the field ( 4 to 6 for hill). The seeds are spaced 0.9-1.8 m apart between rows and 1.5-3 mwithin rows (Omobuwajo *et al*., 2000). Roselle is a short-day photoperiodic plant.

Diseases have been reported as a limiting factor to the production of roselle

worldwide (Ooi and Saleh 1999). The cultivated plants are susceptible to pathogens such as *Phytophthora parasitica*, *Phoma sabdariffae*, *Rhizoctonia solani* (Gomez and Gomez 2008) and *F. oxysporum* (Amusa *et al*., 2005; Agbenin and Ogunlana, 2006). Ooi and Saleh (1999) reported *F. oxysporum* as the principal causal agent of vascular wilt and also the causal agent of stem rot of plants of *H. sabdariffa*. Roselle is attacked by several fungi: *Aecidium garckeanum,Aecidium hibiscisur-attense*, *Alternaria macrospora*, *Cercospora abelmoschi, C. malaysensis, Corynespora cassiicola, Cylindroclad-ium scoparium, Diplodia hibiscina, Fusarium decemcellulare, F. sarcochro-um, F. solani, F. vasinfectum, Guignar-dia hibisci-sabdariffae, Irenopsis molleriana, Leveillula taurica, Microsphaera euphorbiae, Phoma sabdariffae, Phymatotrichum omnivor-um, Phytophthora parasitica, Phytoph-thora terretris, Pythium perniciosum, Rhizocotonia solani, Sclerotinia fuckeliana, S. sclerotiorum, Sclerotium rolfsii* (Khairy, 2001). The bacterium, *Bacillus solanacearum*, has been isolated from roselle. Roselle is seriously attacked by root-knot nematodes: *Meloidogyne arenaria*, M. *incognita acrita* and *M. javanica (*Orwa *et al*., 2009). Among *the insect pests which attack roselle are: Anomis erosa, Chaetocnema spp., Cosmophila erosa, Dysdercus cingulatus, D. poecilus, Drosicha townsendi, Nistora gemella, Phenacoccus hirsutus, Pseudococcus filamentosus and Tectocoris diophthalmus. H. sabdariffa* is severely affected by *Phytophthora [nicotianae var.] parasitica and Rhizoctonia bataticola [Macrophomina phaseolina]* which cause foot and stem and root rot diseases. The powdery mildew *Leveillula taurica* attacks *H. sabdariffa* (Orwa *et al*., 2009).

Roselle has drastically reduced in yield and production due to the attack by diseases.One of the major axes of *Hibiscus* improvement programs consists of the development of resistant genotypes against fungal diseases. There is a paucity of information on the effect of pathogenic microorganism on roselle seed germination and seedling growth in Nigeria.

## MATERIALS AND METHODS

Healthy Seeds were obtained from Plateau State Agricultural Development Program (PADP) Jos for this study. This experiment was carried out at the Green House of the University of Port Harcourt. Ten grams of healthy seeds in sterile conical flasks were inoculated with the organisms isolated from diseased seeds (Nwaukwu and Ataga, 2012). These organisms are the fungi ,*Aspergillus flavus* Link Ex fr, *Aspergillus niger* Van Tieghem, *Fusarium oxysporum* Schlecht, *Penicillium chrysogenum* Thom and *enicillium roqueforti* Thom and bacteria are,*Arthrobacter* sp, *Erwinia* sp, *Lactobacillus* sp and *Corynebacteria* sp. For the fungi, 1.5cm

diameter cork borer was used to pick the test fungi, transferred aseptically into the sterile conical flasks and 10ml sterile distilled water. The conical flasks were shaken vigorously to obtain a homogeneous mixture and then kept in a dark place for 24hours. For the bacterial isolates, each test bacterium was used to make a serial dilution with physiological saline and $10^{-3}$ dilution was used. Exactly 0.1ml of each of the isolated bacterial were inoculated into the conical flask and also shaken for homogeneous mixture. This was also kept in the dark room for 24 hours. The same procedure was applied to the control but the seeds were not inoculated.

The infected and uninfected seeds were aseptically taken to the green house, where they were planted in sterile sandy loamy soil in polythene bags. Three infected seeds were planted per bag. A total of nine test organisms were used and the control for each organism. The treatments were replicated 5 times giving a total of 50 plants. Completely randomized design (CRD) was used as the experimental layout. The bags were well spaced at 1.5m apart. The seeds were watered daily and examined for germination, signs and symptoms growth of disease. The growth parameters measured were leaf number, leaf length, leaf breadth, leaf area and stem length.

The experiment lasted for one month and leaf of samples were taken to the laboratory for re-isolation to confirm the identity of the isolate.

For the seedling growth, 10 healthy seeds each were planted in sterile sandy loam soil in 50 sterile polythene bags for nine test organisms (bacteria and fungi) and the control. Each test organism was replicated five times. The bags were labelled accordingly. The plants were watered daily and after 1 month, plants were inoculated with each test organism at the three leaf stage.

Spores of each fungus were collected from culture in Petri-dishes and the bacteria from the slants. For the bacterial organisms, serial dilution method was used from the $10^{-3}$ dilution where 0.1ml of each of the isolated bacteria was sprayed using the run –off method on 40 plants. For the fungal cultures, $4cm^2$ agar pieces were lifted into a beaker containing 10ml of distilled water. The spores were thoroughly dislodged into the water with a carmel's hair brush and sieved through double layer muslin cloth to remove the hyphae, agar lumps and other impurities. Conidal suspension for inoculating each fungus was standardized at 10, 000 spores per ml, in gelatine-water. The spore suspension was standardized with the aid of a haemocytometer slide. Galatin (0.1%) was used as a sticker. Each isolate in the suspension was used to spray to run-off, with a laboratory sprayer on separate seedlings raised to 2-3 leaf stage in sterile sandy loamy soils, in plastic bags in the green house. A total of fifty seedlings were used for the pathogenicity tests

with each fungal suspension sprayed on each seedling in a bag. After spraying, each plant was then covered with a large sterile polythene bag for 24 hours to maintain about 100% relative humidity. Five polythene bags which were used as control for each organism were sprayed to run-off with sterile distilled water only and covered with polythene bags for 24hours. The plants were watered daily and examined for signs of infection, where symptoms occurred; isolation was again carried out to confirm the identity of the isolates. Analysis of variance (ANOVA) was carried out on all data collected. Bar graphs were ploted and the standard error bar were noted at 95% confidence limit and Fisher least significant difference (F-LSD) at 5% probability level (P=0.05)

**RESULTS**

The effect of *Aspergillus flavus, Aspergillus niger, Fusarium oxyspor-um, Penicillium chrysogenum* and *Penicillium roqueforti*; *Arthrobacter* sp, *Erwinia* sp, *Lactobacillus* sp and *Corynobacteria* sp on germination of *H. sabdariffa* seeds are presented in Fig. 1. There was significant reduction (P=0.05) in germination of seeds treated with the test organisms when compared with the untreated control. *Erwinia* sp caused the highest reduction in germination when compared with other test organisms.

The test organisms caused various symptoms on the leaf of *H. sabdariffa*. *Fusarium. oxysporum* caused dark brown leaf spot, rapid discolouration, wilt, stunted growth, watery rot and necrotic lesions on the leaves. *Penicillium chrysogenum* caused round leaf spot, chlorosis and rot. *Aspergillus niger* caused dark leaf spot, dry patches on leaves and black rot. *P.roqueforti* caused leaf spot. *A. flavus* caused yellow rot. *Arthrobacter* sp. caused leaf blight. *Erwinia* sp. caused fire blight and bacterial leaf spot. *Lactobacillus* sp caused scanty leaf spot. *Corynebacterium* sp. caused Scanty yellow leaf patches. The control experiment had no symptom on the leaves of the plant. There was significant increase (P=0.05) in the number of infected leaves after 2 weeks of germination (Fig 2) when compared to the uninfected control. There was significant reduction (P=0.05) in the leaf length, leaf area and stem height at 3 weeks after planting when compared to the uninfected control (Fig 3-5).

The mean number of infected leaves ( disease incidence) 2weeks after germination showed that *Fusarium oxysporum* and *Corynebacterium* sp. had the highest mean number of infected leaves while *A. flavus, Erwinia* sp., *P. chrysogenum, Lactobacillus* sp., *A. niger , Arthrobacter* sp. and *P. roqueforti* ranked lower in descending orders. However, the control had no number of infected leaves but the micro-organisms had significant reduction (P=0.05) on the leaf length 3 weeks after planting. *Erwinia* sp. had the least mean

reduction on the leaf length followed by *Corynebacterium* sp. *Fusarium oxysporum* , *P. chrysogenum A. flavus, A. niger Lactobacillus* sp. and *Arthrobacter* sp., in ascending order. Control had the highest leaf length when compared to the treated plants

There was a significant reduction (P=0.05) in the leaf area and stem length at 3weeks after planting (Figs. 4 and 5) of the seedlings infected with fungi and bacteria organisms when compared to the uninfected control. *Erwinia sp*. caused the least reduction in leaf area(5.0cm$^2$), followed by *Corynobacteria* sp. (7.0cm$^2$), *Fusarium oxysporum* (9.0cm$^2$), *Penicillium chrysogenum* and *Aspergillus niger* (10.0cm$^2$), *Aspergillus flavus* (11cm$^2$), *Lactobacillus* sp. (12cm$^2$), *Arthrobacter* sp. (14cm$^2$) and *Penicillium roqueforti* (20cm$^2$) when compared with the control plant (29cm$^2$). Also, *Fusarium oxysporum* caused the highest reduction of mean length of stem (16cm),followed by *Erwinia* sp (16.5cm), *Aspergillus niger* (17cm), *Penicillium chrysogenum* (18cm), *Arthrobacter* sp. (20cm), *Corynobact-eria* sp. (22cm), *Lactobacillus* sp. (24cm) and *Penicillium roqueforti* (25cm), when compared to the control (30cm).

## DISCUSSION

The effects of the individual micro-organisms (*F. oxysporum P. chrysoge-num, A. niger P.roqueforti A. flavus Arthrobacter* sp. *Corynebacterium* sp.) on seed germination and seedling growth showed diverse degree of inhibition on the growth parameters. *Erwinia sp.* had the least reduction (20%) effect on germination when compared with the control plant which showed 100% yield. While *Lactobacillus sp.* and *Coryne-bacterium sp*. had the next reduction of 20.4%, others are *Arthrobacter sp.* (25%) *F. oxysporum* (20.8%), *A. flavus* (30.0%) *P. chrysogenum A. niger* (30.2%) *P.roqueforti* (40%). Nahed (2008) in his work on the effect of filtrates of pathogenic fungi of soybean on seed germination and seedling parameters, obtained similar results that filtrates of *A. niger, F. culmorium, Pen-icillium* sp. and *R. solani* inhibited seed germination and seedling development of soybean. He further reported that the fungi produced toxic metabolites in the media in which they were grown which inhibited and reduced the percentage of seed germination and also retarded seedling growth. Similarly, Umechuruba and Nwachukwu (1997) reported that *A. flavus, A. niger, F. moniliforme*,and *Penicillium* sp. produced various types of toxic metabolites which are known to reduce germination and seedling development of African yam bean seeds.

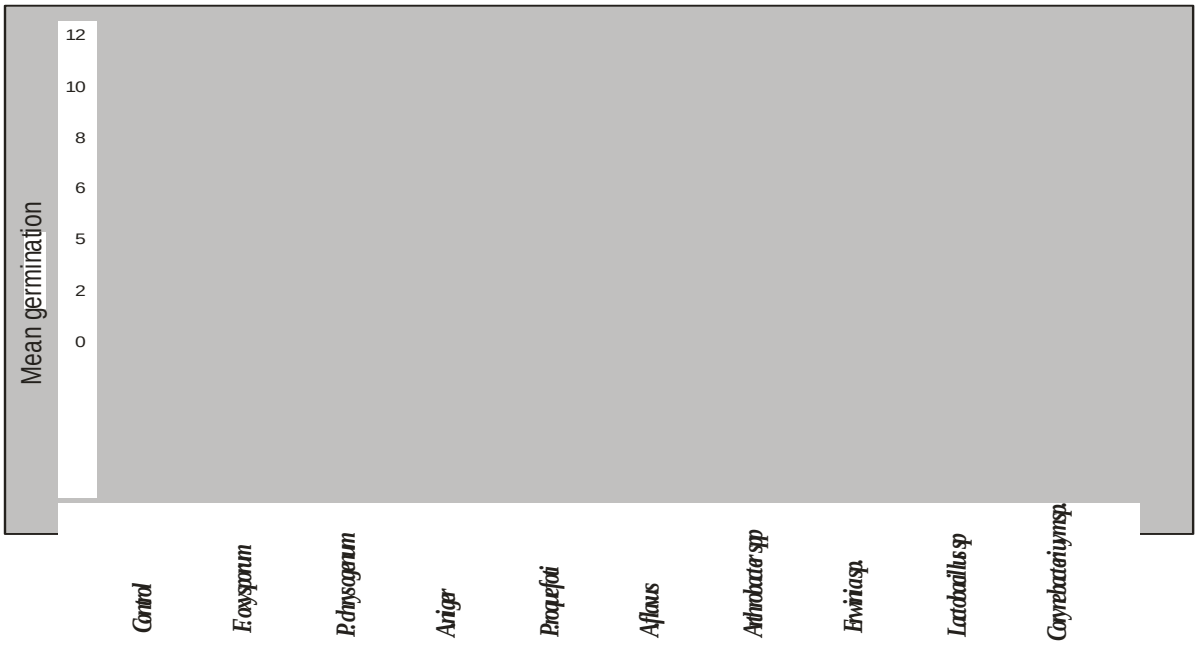


**Fig 1: Effect of microorganism on germination of *H.sabdariffa* seed**
I = Standard error (P=0.05)

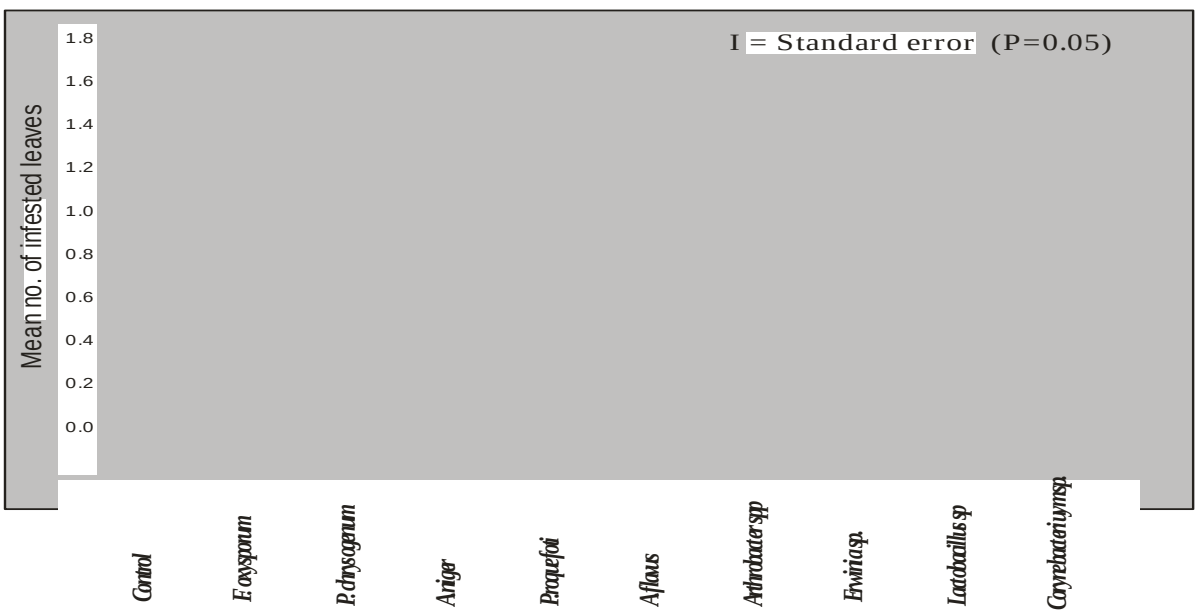


**Fig 2: Number of infected leaves (disease incidence) 2wks after germination**

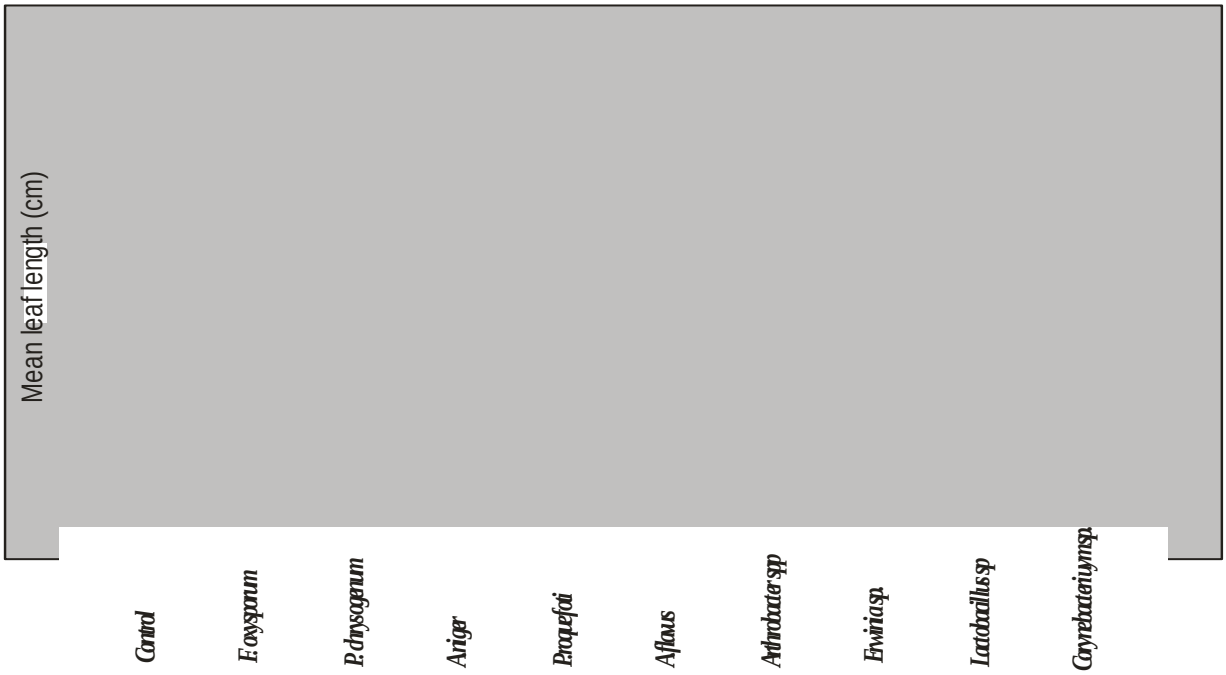


**Fig 3: Effects of microoganisms on leaf length, 3 weeks after planting**
I = Standard error (P=0.05)

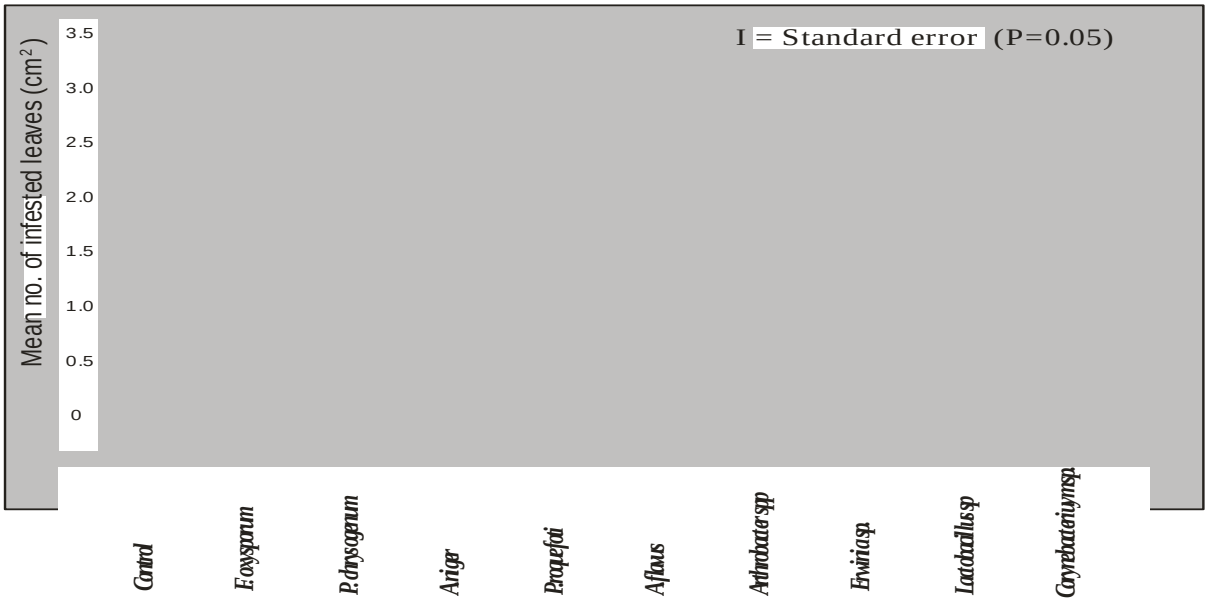


**Fig 4: Effects of microoganisms on leaf area, 3 weeks after planting**

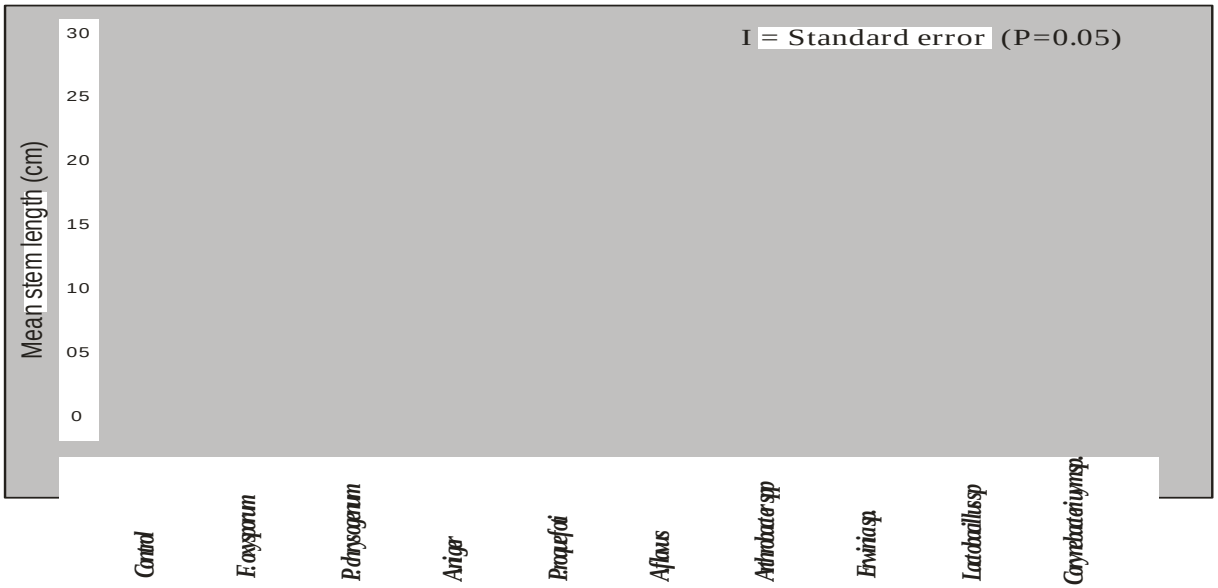


**Fig 5: Effects of microoganisms on length of stem, 3 weeks after planting**

*Aspergillus flavus*, *A niger*, *F. oxysporum*, *Penicillium* and *P roque-forti*; *Arthrobacter* sp., *Erwinia* sp, *Lacto-bacillus* sp and *Corynobacteria* sp. organisms caused various symptoms on the leaf of *H. sabdariffa*. *Fusarium oxysporum* caused dark brown leaf spot, rapid discolouration, wilt, stunted growth, watery rot and necrotic lesions on the leaves. *P chrysogenum* caused round leaf spot, chlorosis and rot. *A niger* caused dark leaf spot, dry patches on leaves and black rot. *P.roqueforti* caused leaf spot. *Aspergillu flavus* caused yellow rot. *Arthrobacter* sp. caused leaf blight. *Erwinia* sp. caused fire blight and bacterial leaf spot; *Lactobacillus* sp caused scanty leaf spot and *Corynebacterium* sp. caused scanty yellow leaf patches. However, the control had no symptoms on the leaves. Oyewale (2006) in his work on fungal diseases of sweet potato reported that foliar diseases of sweet potato are mainly leaf spots and powdery mildew which were mainly caused by *F.* oxysporum. Amienyo (2008) implicated *A niger* and *F oxysporum* to be the most notorious organisms that cause reduction in the seedling growth of sweet patato. According to the report of Manners (1993), blights are caused by pathogens which kill cells with which they come in close contact and become generally distributed either by spreading through the plant or by means of multiple infections.

It could be concluded that *F. oxysporum* and *Erwinia* sp. isolates caused more harm to both the seed germination and seedling growth. They could cause diseases that retard the

growth of the plant consequently reducing the yield and production of the Yakwa plant.

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