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Influence of the Number of Watering and Fungicide Treatments on the Development of *Phytophthora colocasiae* (Racib) on Cocoyam (*Xanthosoma sagittifolium*) and Taro (*Colocasia esculenta*) Greenhouse in Cameroon

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Three research areas have been explored: the evaluation of the fungus *P. colocasiae* attacks on cocoyam (*Xanthosoma sagitifolium*) and taro (*Colocasia esculenta*), the appreciation of the influence of moisture on the development of the fungus and evaluating the effectiveness of some fungicides on the fungus *P. colocasiae*. Four blocks each containing 30 feet of cocoyam and taro 30 feet constituted the test. These blocks were differentiated by daily watering frequency. The plants were then treated with systemic fungicides after removal of infected leaves. The results show that the humidity is a factor that favours the development of the fungus *P. colocasiae*. Block 3 with a higher frequency of watering experienced more attacks compared to blocks 1 and 2 that were less watered and therefore a lower humidity. The frequency of watering interaction and number of attacked plants was highly significant (<5%). The disease develops in both the taro and cocoyam but with a higher rate of attacks on taro. The application of systemic fungicides on plants after extraction of infected leaves has not stopped the spread

Introduction

Food crops occupy an important place in the cameroonian economy and the fight against food insecurity. Of these crops, taro (*Colocasia esculenta* L. Schott) is one. It is a staple food for people in some regions of Cameroon with regard to some of its nutritional qualities. Indeed, many taro varieties of tubers those provide highly digestible starches, attractive for patients and infants (Wang, 1983). Their leaves are eaten as greens. They have high levels of thiamine, which nowadays is an advantage because the leaves enable people to consume refined carbohydrates. They are also an excellent source of vitamin C, niacin and riboflavin which are all vitamins necessary for the body (Onwueme, 1999).

C. esculenta is a monocotyledonous herbaceous plant, cultivated in tropical and subtropical regions. Native to parts of Southeast Asia and the Pacific They (Jianchu *et al*., 2001), it was introduced in Africa about 2000 years ago by the African East Coast (TPI, 2000-2004). Today, it is one of the five largest world tubers alongside cassava, potato, sweet potato and yam (FAO, 2010; Tewodros, 2013).

Cameroon and particularly in the western region, taro is a cultural food. It is a sacred and honored food. It is difficult, if not unthinkable to organize a ceremony in Bamileke country without taro dishes. However, several factors limit its production and mainly taro blight that is currently the leading cause of lower productivity.

This disease appeared in Cameroon in 2010 (Fontem *et al*., 2011). His appearance caused a general panic among producers, the abandonment of cultivated land and therefore the scarcity of tubers and leaves on the local market. This is a fungal disease and its causal agent *Phytophthora colocasiae* (Racib), (Ghosh *et al*., 1991). It causes circular water-soaked lesions, purple to brown on leaves.

P. colocasiae is known to infect taro (C. esculenta, C. esculenta var. *Globulifera*) and some cultivars monkey or tamu (*Alocasia macrorrhiza*). No illnesses developed when this fungus was sprayed on cocoyam (*Xanthosoma brasiliense*) or other species of *Xanthosoma* (Lokesh *et al*., 2014; Debon & Boula, 1992).

Mildew was found in all of the taro growing areas. The first symptoms of infection are characterized by the appearance of black circular spots on leaves and petioles. It destroys the parts of the leaf responsible for photosynthesis reducing its activity (Cox and Kasimani, 1990a). Younger leaves on the adult plant are not affected by the disease. This is a condition that develops rapidly and can cause premature leaf death. Mildew usually appears during the rainy season, with high humidity, relatively low night temperatures (20-24 ° C) and daytime temperatures between 25 and 28 ° C.

Under these conditions, the disease spreads very quickly and within a week all the plants of the field may be severely affected. The space between the taro plants also is another factor that may influence the spread of the disease. It spreads rapidly in densely planted plots (Ivancic & Lebot, 2000). Infection often starts on the lobes and the sides of the sheet where the water accumulates. The spots enlarge, take irregular shapes and become dark brown edged with yellow. The initially specks give rise to secondary infections and soon after, the leaf blade collapses and dies. This disease has caused considerable losses in Cameroon, reducing the yield of over 70% (Fontem *et al*., 2011). In the Solomon Islands, for example, the taro cultivation had to be partly replaced by sweet potato lack of effective treatment (Cox and Kasimani, 1990b). The disease is present in Africa, India, Southeast Asia and several Pacific countries such as the Hawaiian Islands, the Solomon Islands, Papua New Guinea etc. (Trujillo, 1965; Gollifer *et al*., 1980; Jackson, 2001; Thankappan, 1985).

Regular removal of diseased leaves in the field does not reduce the rate of infection during rainy periods. The use of systemic fungicides such as Ridomil at regular intervals can stop the development of the disease (Das, 1997; Ghosh, 1991; Cox and Kasimani, 1990b). The best option is to fight the spread and use of resistant cultivars (Misra, 2000).

Cocoyam is an herbaceous monocot native to South America. The practice of this culture dates of the pre-Columbian era when it was introduced in Africa in the 1840 (Doku, 1980). Its cultivation is widespread and covers the central and South America, Central and West Africa, Oceania, New Caledonia and the South East Asia (Bown, 2000). In Cameroon it is grown from the high western plateau to the low southern forest land.

Cocoyam belongs to the series of superovariées. It is part of the family *Aracea*, subfamily *Colocasioideae*, the *Xanthosoma* genus of nearly 2500 species of which 57 are cultivated (Bown, 2000). The species grown in Cameroon is *Xanthosoma sagittifolium*.

Reproduction is mainly done vegetatively and planting material consists of tubercle fragment or whole tubers (Aguegia *et al*., 2000). Sexual propagation of cocoyam exists, but it is rare. It allows the recombination of genes from different species to create new varieties. From the study of biological characteristics of *P. Colocasiae* we will identify specific traffic patterns, associations and antagonisms of the different strains encountered in Cameroon, to optimize its IPM. Varietal improvement offers genetic control as an alternative to chemical control. To this end, one way conducive to improving taro performance is the creation of varieties resistant to *P. colocasiae* (Monkam *et al*., 2015; Yared Dagne, 2014).

The overall objective of our study is to contribute to the improvement of the production of taro by reducing losses and damage caused by fungi in general and particularly taro blight. This overall target has been split into several specific objectives include: assessing the fungus attacks on cocoyam and taro, appreciate the influence of moisture on the level of development of the fungus and evaluate the effectiveness of some fungicides the mushroom.

Materials and Methods

Plant material

The seed used was solely composed of local varieties of taro and cocoyam (red and white). The seedlings were taken in the fields of local producers in the city of Batouri. These seedlings were previously sterilized in an aqueous hypochlorite solution and sodium chloride 2.6% available chlorine for 30 minutes and then rinsed with water before being cultured.

Isolation and purification of *P. Colocasiae*

Infected leaves of the taro blight were collected in the fields of local producers and swept the laboratory of Plant Biology and Physiology, University of Douala, Cameroon. They were cut into fragments of approximately 2 mm^2 at the level of pathogen growth front before being disinfected superficially in a sodium hypochlorite solution at 5% for 2 min. After three rinses with sterilized distilled water (SDW), the fragments were dried over hydrophilic paper then applied at a rate of four fragments per plate on medium gelled V8 cultivation supplemented with a penicillin compounds antibiotic solution (250mg / l), ampicillin (250 mg / l) and nystatin (20 mg / l) (Djeugap *et al*., 2009; Tsopmbeng *et al*., 2012). After three days of incubation in the laboratory at a temperature of 23 ± 1 ° C, colonies of the pathogen, visible around fragments were picked and subcultured into new petri dishes containing PDA culture medium. This process was repeated several times until obtaining pure cultures of *P. colocasiae* then identified under the ordinary microscope based on morphological characteristics of the mycelium (not septate) and fruiting bodies (sporangia) as described by Brooks (2005) and Scot *et al.*, (2011).

Experimental set-up

Four blocks each containing 60 feet (30 feet and 30 feet cocoyam or taro) were set up. After clearing and cleaning of the plot, 10 cm deep holes were drilled within which the seedlings cocoyam and taro were planted. The blocks were then covered with clear plastic, in order to avoid the influence of rainfall (Fig. 1).

Layout and principle of the blocks

To measure the influence of moisture on the development of the disease, four blocks were treated as follows:

- In the third block, cocoyam and taro plants were watered three times a day (morning, afternoon and evening). This watering was done throughout the plant;

- In the second block, the plants were watered twice daily (morning and evening);

- In the first block, the plants were watered once a day while the last block was used as a control and was not watered (Fig. 2).

Plant contamination mode and disease assessment

Pure cultures of *P. colocasiae* were filed under a sunny day on the young leaves of the taro plants and cocoyam our test allowing contamination.

Fungal diseases symptoms were identified by visual diagnostic. It recognized the presence of the fungus on the plant with dark brown or green spots present on the leaf rot of leaves and stems ones. The number of plants with these symptoms was recorded all the last three days of the week. Obviously the leaves were no longer counted (Ambang *et al.*, 2016). This allowed us to calculate the incidence of formula Tchoumakov (1990):

$$I = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Phytosanitary treatment plants

To test the efficacy of fungicides against the disease development, twenty plants per block (10 taro and 10 cocoyam) were selected for treatment in Ridomil gold plus 66 wp (60g / kg metalaxyl M and 600g / copper kg d'oxyde) and cacaocobre 50 wg (50% pure copper metal) all are the systemic fungicides.

The solution was obtained by diluting 50 g of each fungicide in 15 liters of drinking water. The treatment is carried out during a quiet time (no wind) and sunny day. These chemical treatments were done once a week and after extraction of diseased leaves. Observations were made regularly to assess the response and behaviour of the treated plants. The experiment lasted 04 months.

Data analysis

Data was analyzed using the computer program, software analysis system (SAS), which uses the analysis of variance (ANOVA) and the test of Student-Newman-Keuls at 5% to compare the means.

Whenever there was a significant difference between the means, the least significant difference (LSD) method was used to separate them.

Results and Discussion

Effect of watering frequency

After infestation of plants and watering, it appears from the results (table 1) that the amount of moisture influences the development of the fungus: 41 cocoyam plants and averagely 60 taro were infested after 3 weeks of watering for block 3 compared to 6 infested plants for the control block. We also find that taro is more susceptible to the fungus *P. colocasia* compared to cocoyam respectively 26/30 and 15/30 infested plants at block 3, 14/30 and 10/30 at block 2. The plants are mostly infected during their first week of growth (20, 13 and 8 respectively for the first, second and third week).

Change in number of infected Taro plant depending on the frequency of watering

The frequency of watering is highly significant (P <0.05) on the number of infected taro plants. Over watered, the number of infected plants evolves. The fungus has grown significantly in the block 3 (26/30) which was watered three times a day compared to blocks 2 (14/30) and block 1 (6/30) that were sprayed respectively twice and once a day (fig.3).

Change in number of infected cocoyam plant depending on the frequency of watering

Fig. 4 shows that frequency of watering positively influence the development of the fungus (P <0.05). However, comparison of average pair wise of Turkey shows no significant difference between the pairs: (block 3, block 2), (Block 3, Block 1), (Block 2, Block 1), (Block 2, control) and (block 1, control), but significant between block 3 and control.
Disease has flourished on the most watered blocks (block No3: 3times / day and block 2: 2 times / day) compared to those who were less watered (block 1: 1 time / day and control 0 time / day).

Changes in the number of infected plants depending on the frequency of watering and plant type interaction

The fungus attack taro as well as cocoyam, but taro is more sensitive and the difference is significant (P <05). In block 3, for example, we observed 86.67% of taro plants with 50% of plants against cocoyam and overall 40% of taro plants infected 31.67% against cocoyam (Fig.5).

Whether taro or cocoyam, watering frequency has a positive influence on the development of the fungus (P <0.05). The disease develops much more on the most watered blocks. The fungus grew much on Block 3 which was washed three times, 41 plants attacked on 60 one percentage 68.33%. Block 2 which was watered twice daily had an attack rate of 40% while the block 1 watered once a day experienced a 25% attack rate and the block controls 10%.

Effectiveness of chemical treatments on the fungus

After extracting the infected leaves, the plants were treated with ridomil and cacaocobre (systemic fungicides). We found that the average number of plants per week attacked by the fungus was growing 4, 7 and 9 respectively for the first, second and third week after the chemical treatment. By cons we noticed disease stability from the second week for cocoyam; 2 infected plants in an average week (Fig. 6).

It appears from these data that the chemical treatment has not stopped the development of the disease. The fungus continued to expand much on taro plants and less on those of cocoyam.

On the block who received two waterings a day, we obtained similar results to the block 3 but with less intensity (Fig. 7). By cons, we noticed a stable disease during three weeks in block 1, unlike the blocks 2 and 3. It also notes a relatively high number of cocoyam plants affected by the disease compared to taro (Fig. 8).

Analysis of variance in the average number of infected plants after chemical treatment shows an insignificant effect on the development of the disease (P> 0.05). This confirms that the chemical treatment does not influence the development of disease.

The results obtained in this study show that humidity is a factor that influences the development of the fungus *P. colocasiae* (Cox and Kasimani, 1990a).

It is for this reason that the rate of attacks was higher in block 3 watered three times a day; 68.33% compared to the block 2, 1 and control which were 40%, 25% and 10% of attacks. Which is also normal because fungi generally grow best in the presence of moisture. The fungal spores rapidly germinate in the presence of water (Cox and Kasimani, 1990b).

Table.1 Number of plants attacked by species depending watering frequencies in each block

Block	watering frequency (times / day)	Species	average number of plants attacked a week after infestation leaves												
			1st week				2nd week				3th week				Total average for weeks
			5 day	6 day	7 day	X	12 day	13 day	14 day	X	19 day	20 day	21 day	X	
3	3	Taro	14	13	9	**12**	8	7	9	**8**	7	7	4	**6**	**26**
		cocoyam	9	7	8	**8**	7	5	3	**5**	3	2	1	**2**	**15**
		total	23	20	17	**20**	15	12	12	**13**	10	9	5	**8**	**41**
2	2	Taro	8	6	4	**6**	5	5	5	**5**	4	1	1	**3**	**14**
		cocoyam	6	7	2	**5**	2	2	2	**3**	1	1	0	**2**	**10**
		total	14	13	6	**11**	7	7	7	**8**	5	2	1	**5**	**24**
1	1	Taro	6	4	2	**4**	3	2	1	**2**	0	0	0	**0**	**6**
		cocoyam	7	6	2	**5**	2	2	2	**2**	2	2	2	**2**	**9**
		total	13	10	4	**9**	5	4	3	**4**	2	2	2	**2**	**15**
Control	0	Taro	4	1	1	**2**	0	0	0	**0**	0	0	0	**0**	**2**
		cocoyam	4	3	2	**3**	1	0	0	**1**	0	0	0	**0**	**4**
		total	8	4	3	**5**	1	0	0	**1**	0	0	0	**0**	**6**
general total			58	47	30	**45**	28	23	22	**26**	17	13	8	**15**	**86**

Fig.1 Block containing plants covered by transparent plastic (F1- ridomil, F2- cacaocobre)

F1

F2

Fig.2 Experimental design, M- cocoyam, T-taro, F1-ridomil, F2- cacaocobre

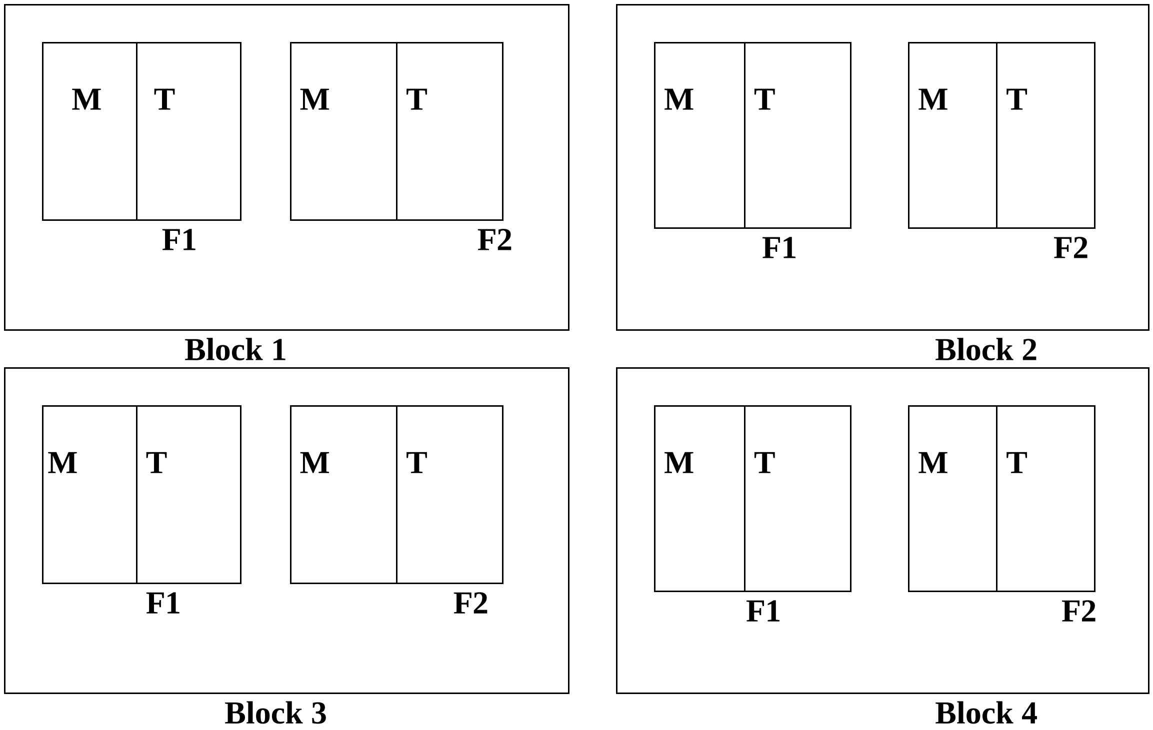


Fig.3 Changes per week on the average number of taro plants infected with *P. colocasiae* depending on the frequency of watering.

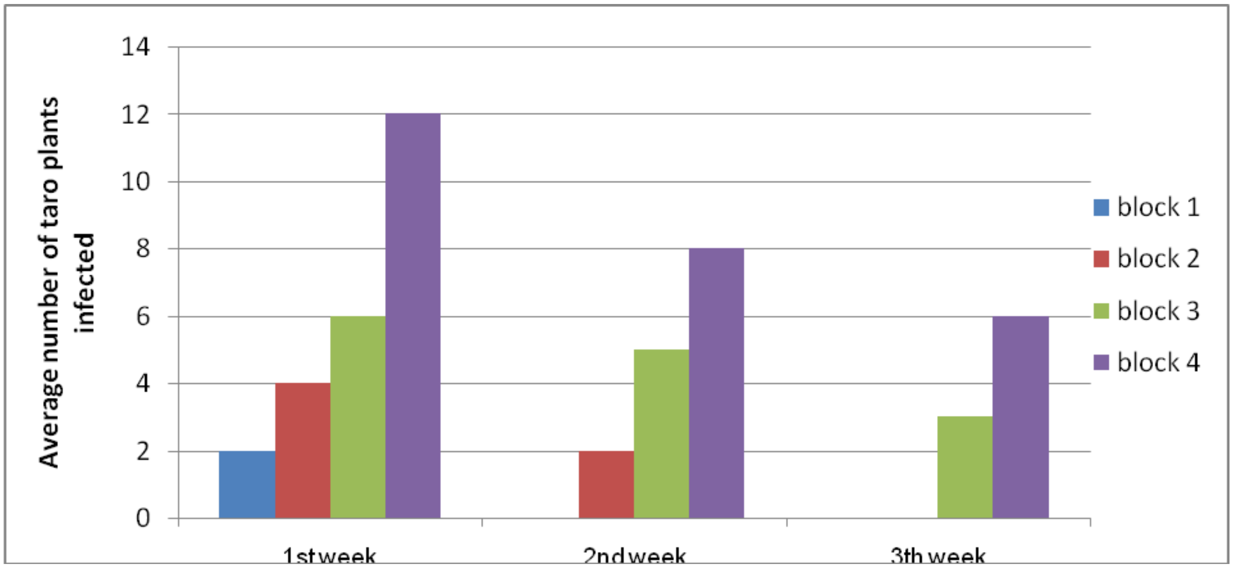


Fig.4 Changes per week on the average number of cocoyam plants infected with *P. colocasiae* depending on the frequency of watering

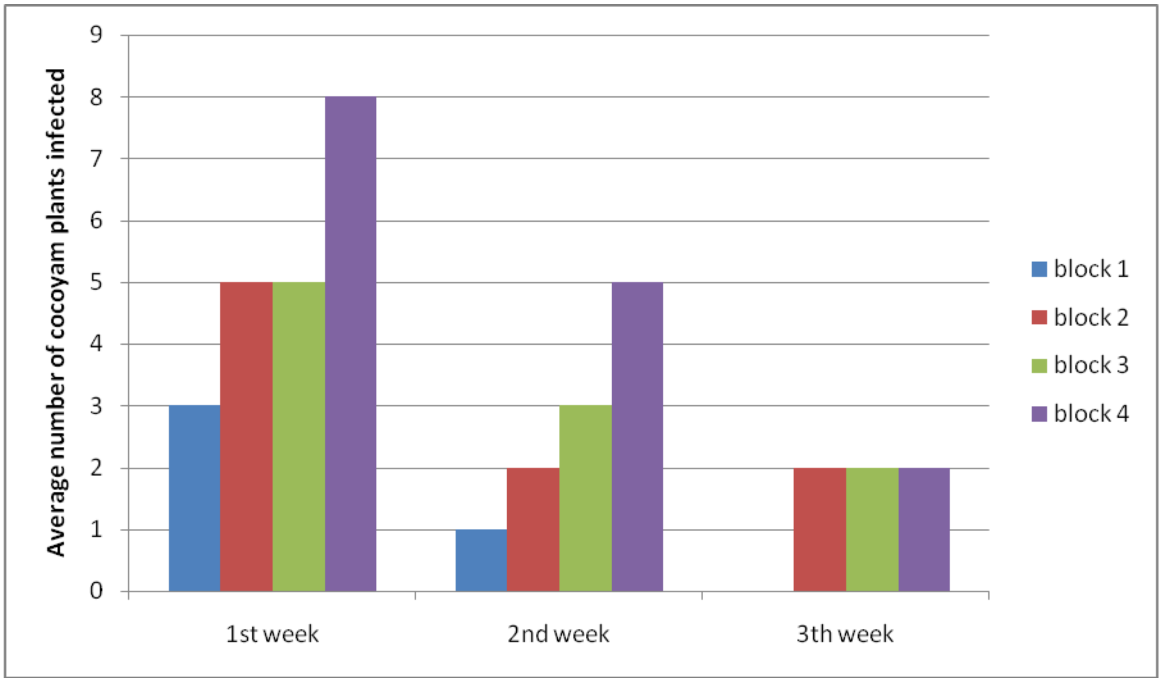


Fig.5 Number of cocoyam and taro plants infected depending on the frequency of watering in each block

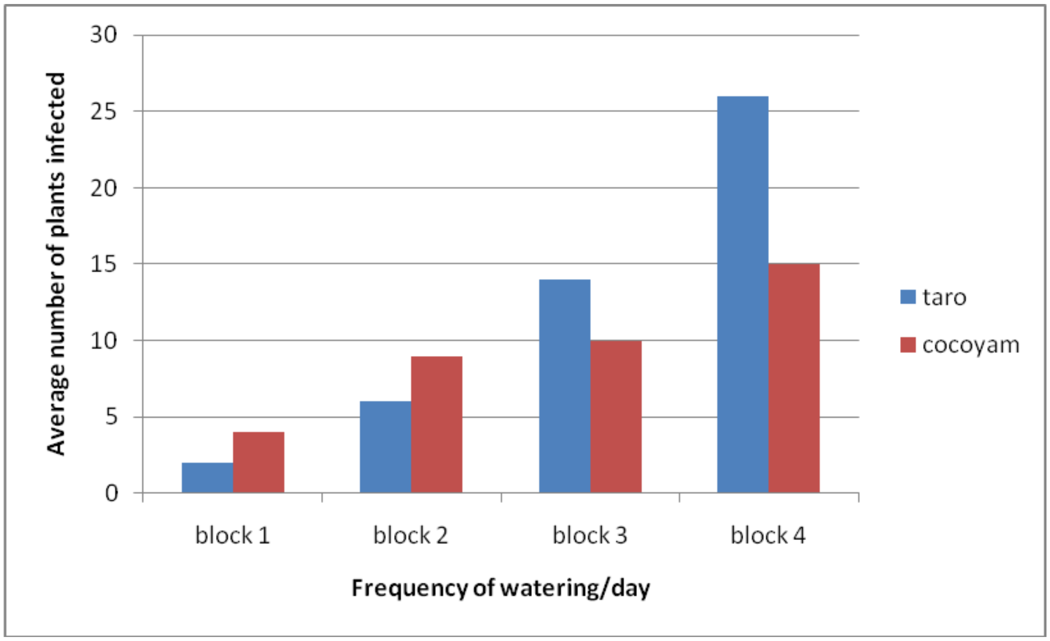


Fig.6 Effect of chemical treatment on Block 3

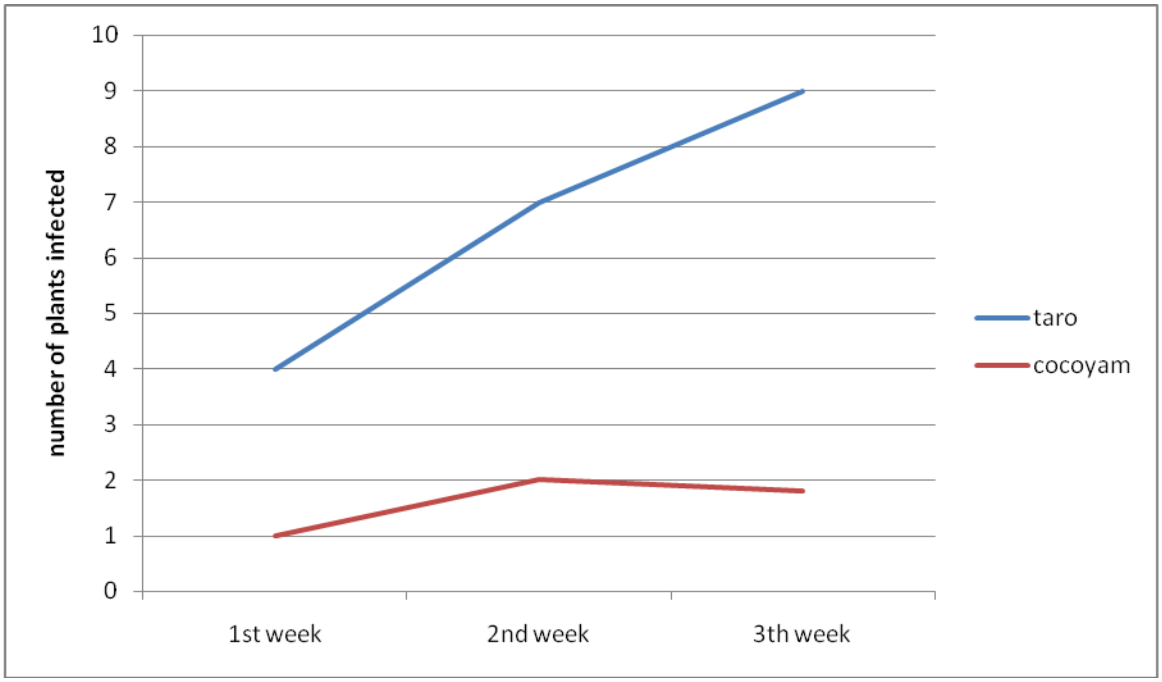


Fig.7 Effect of chemical treatments on Block 2

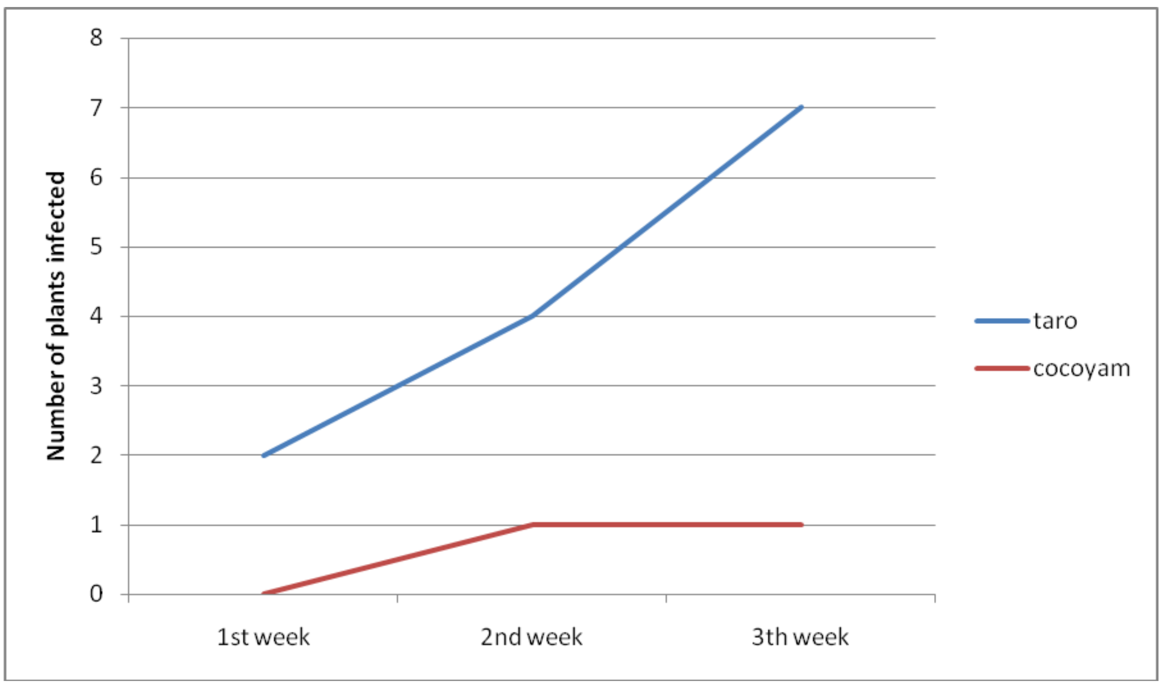
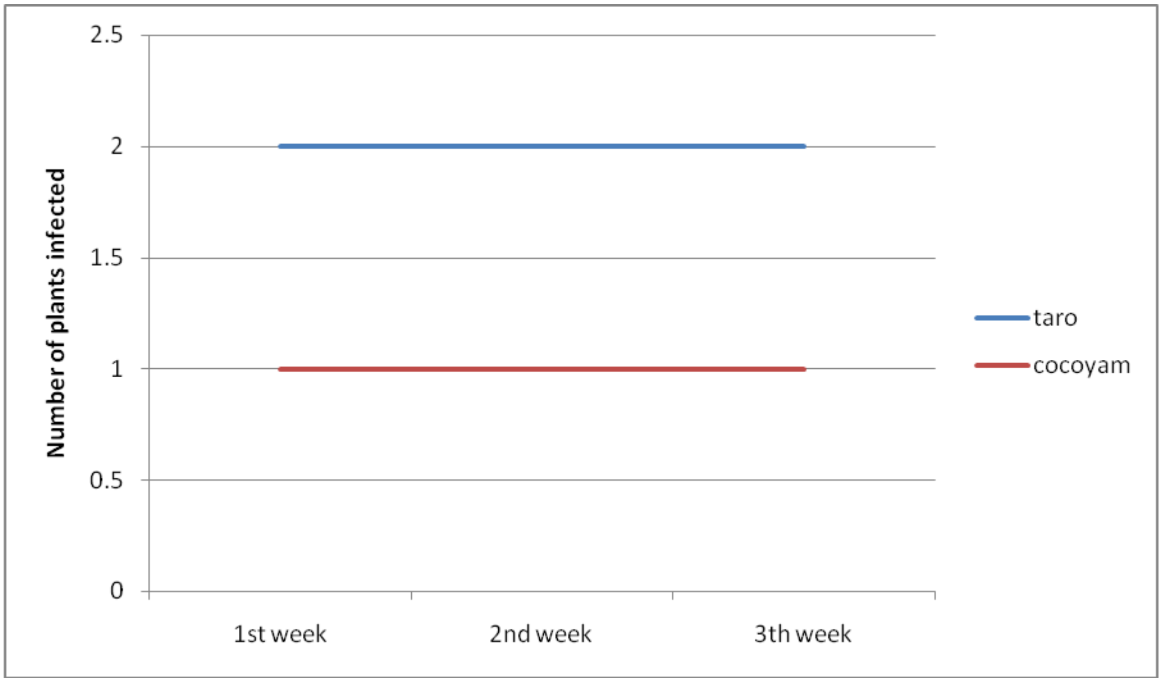


Fig.8 Effect of chemical treatments on Block 1



The fungus grows on both the cocoyam and taro. However, most researches show that the fungus causes damage especially on taro leaves (Cox and Kasimani, 1990a; Ivancic and Lebot, 2000) and a recent study in Karnataka in India has revealed no illness when this fungus was sprayed on cocoyam (*Xanthosoma brasiliense*) or other species of *Xanthosoma* (Lokesh *et al*., 2014). But in this study, we found that *P. colocasiae* also develops on the leaves of cocoyam (50% of attacks observed on cocoyam in block 3 against 86.66% for taro and 33.33% in the block 2 against 46.66% for taro). This could be explained by the fact that both plants are from the same family and therefore likely to have the same parasites or else the strain identified in India is genetically different from the one existing in Cameroon. However, statistical analysis of variance revealed that the watering rate has no significant effect on the development of the disease as regards to cocoyam. However, comparison of average pair wise of Turkey shows no significant difference between the pairs: (block 3, block 2), (Block 3, Block 1), (Block 2, Block 1), (Block 2, control) and (block 1, control) but significant between the block 3 and the control. This means that cocoyam *P. colocasiae* remains less tolerant compared to taro.

The application of fungicides on plants after removal of diseased leaves did not prevent the spread of the disease. According to Anonymous, (2007) no cases of disease caused by *P. colocasiae* were reported on the species of *Xanthosoma* after spraying fungicides. Similarly, Das (1997), Ghosh and Pan (1991), believe that the application of systemic fungicides at regular intervals such as ridomil allows control of the disease. However, we observed in this study despite the extraction of cocoyam leaves the already affected by the disease and after chemical spray plants with ridomil, the fungus has continued its development. The results of the analysis of variances in the number of taro and cocoyam plants infected after chemical treatment confirmed this result, since it has not had a significant effect. The ineffectiveness of chemical treatments to stop the development of the fungus could be explained partly by the fact that the fungus *P. colocasiae* have undergone genetic mutations certainly because of the repeated use of these fungicides have therefore developed resistors (Fry, 1982). This has made the fungicide ineffective against this fungus, on the other hand this inefficiency of chemical treatment could also be explained by the fact that the fungus spores would have already been registered on healthy leaves long before the application of systemic fungicides on plants and would have continued their development. It would be desirable for better control of the disease to remove diseased leaves (or just the infected part) at the onset of symptoms to prevent the spread of spores to healthy leaves (Jackson, 2001). Ivancic and Lebot (2000) classified the taro cultivars into three groups based on water needs: the cultivars adapted to well-watered soil, irrigated or the swamps; intermediate cultivars adapted to moderately watered soils; and cultivars adapted to relatively watered soils. We can therefore think that the cultivars used in this study are not very suitable for very watered soils. This would explain the high percentage of diseased plants in the block 3.

In conclusion, the evaluation of the fungus *P. colocasiae* attacks on cocoyam (*Xanthosoma sagitifolium*) and taro (*Colocasia esculenta*), the appreciation of the influence of moisture on the level of development of the fungus and evaluation of effectiveness of some fungicides on the fungus *P. colocasia* are the three areas of research that have been the subject of our study.

The results obtained and presented here show that the fungus attack as well as taro cocoyam but the latter is more sensitive than cocoyam (40% of attacks against cocoyam 31.67% of attacks on taro). The humidity has a great influence on the development of the fungus. Block 3 which was watered three times a day had more attacks (68.33%) compared to the block 2 (40%), block 1 (25%) and control (10%) respectively watered 2, 1 and 0 times day. This confirms that the humidity favours the development of the fungus. The interaction number of watering and percentage of attacks proved highly significant (P <5%). The application of systemic fungicides even after removal of infested parts has not stopped the spread of the disease. The fungus has continued to grow both in taro and in cocoyams.

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