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Influence of phosphorous acid application on the accumulation of total phenolic compounds in coconut husk (*Cocos nucifera* Linn.)

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Abstract

One mechanism used by coconut plant to protect itself against *Phytophthorakatsurae* is linked to total polyphenols production. This study aimed to investigate the impact of phosphorous acid plant treatment on the production of total polyphenols in coconuthusk, as part of chemical control. The study was conducted on two coconuts cultivars (EGD and PB 121$^+$) with four doses of phosphorous acid [Control, 2.8 g (TA), 5.6 g (TB), 11.2 g (TC)]. At each sampling, the husks were processed and extracts were prepared for total polyphenols assays. There was significant difference between EGD and PB 121$^+$ total polyphenols production ($p<0.001$). The interaction between coconut variety and phosphorous acid doses was also significant. The interaction EGD and TC had the highest total polyphenols accumulation of 4838.5 µg/g of fresh weight (FW). For PB121$^+$, the highest total polyphenols accumulation of 6433.71 µg/g FW was obtained from the interaction between PB121$^+$ and To. From this observation, it could be statedthat phosphorous acid only triggers the treated plantdefense mechanisms to produce total phenolic compounds when attacked by a pathogen.

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Introduction

Coconut palm is a tropical regions perennial plant. Its global area of production is estimated to 11000000 ha (Van der Vossen and Chipungahelo, 2007). In Ivory Coast, its cultivation area covers 50000 ha (Konan *et al.*, 2006), mostly located on the coastal zone (Zakra, 1997). People use it for various purposes including food, cosmetics, craft industry and pharmacopoeia. It also provides livelihood to nearly 12500 Ivorian households (Assa*et al.*, 2006). However, the development of this plant is limited, among others, by attacks of pests and pathogens such as nematodes, phytoplasma, viruses, viroids, fungi and Stramenopiles. They attack almost all organs of the plant like the roots, trunk, leaves and nuts (De Taffin, 1993; Mariau, 1999). The important threats are Phytoplasmacausing coconut Lethal Yellowing and Stramenopiles responsible of *Phytophthora* caused diseases (Mariau, 1999; Oropeza*et al.*, 2010). In Ivory Coast, *Phytophthorakatsurae* causes coconuts immature falling or heart rot, resulting in death of the coconut tree (Renard and Quillec, 1984; Blaha *et al.*, 1994). An infected tree losses can reach 75 % of its annual production, and 50 % of yield lossis possible in plantation (Bennet *et al.*, 1984). Against *Phytophthorakatsurae,* several methods are recommended namely prophylactic, genetic and chemical controls. Mainly, the chemical controluses phosphorous acid, as a fungicide.When the product is injected into the trunk of the coconut trees, it leads to immediate action on the parasite, reducing about 70% of the damage caused by the pathogen (Allou, 1992). A part from this direct action, the fungicide initiates an indirect action including changing of metabolism through induction and stimulation of certain enzymes activity such as polyphenol oxidases (PPO) and phenolic compounds (Benhamou, 2009) that play an antimicrobial role for plants. In fact, they protect plants against insects and pathogens attacks. However, in coconut, the biochemical mechanisms used after treatment with phosphorous acid have been very little studied. Therefore, this study, conducted in the general framework of chemical control of *Phytophthorakatsurae*, was aimed to assess the impact of phosphorous acid treatment of coconut palms on the synthesis and accumulation of total polyphenols in coconut husk.

Materials and methods

Plant material

Two varieties of coconut trees, the improved hybrid PB 121^{+} and the variety Equatorial Green Dwarf (EGD) were used. These two varieties were used because of their susceptibility to the disease caused by the fungus *Phytophthorakatsurae* (Bourdeix *et al.*, 2005).

Chemical reagents

The fungicide used was phosphorous acid (H_3PO_3) in 98.5% crystals (Panreac, Spain). With a molecular weight of 82 g.mol^{-1} and a density of 1.65 g.cm^{-3}, this product was colorless highly soluble in water (Anonymous, 2015). The Follin-Ciocalteu reagent (12 N) was used to assay the total phenolic compound present in the husk.

Treatment of coconut trees

Fifteen coconut trees were used per variety and per treatment. Four doses of phosphorous acid were applied: T0 = Control (no product applied), TA = 2.8 g of active material of phosphorous acid in 5 ml of water, TB = 5.6 g of active material of phosphorous acid in 5 ml of distilled water and TC = 11.2 g of active material of phosphorous acid in 7.5 ml of distilled water, to allow the fungicide to be dissolved easily.

An oblique hole was made by a self-propelled medium drill for TA and TB treatments to a depth of about 12 cm in the trunk and 50 cm above ground. For both treatments, the total volume of each product wasintroduced in a single hole. Due to the total volume of TC product that could not be absorbed by a single hole, two holes located on the same side were pierced. These holes were resealed by a wooden dowelcoated with grease and motor oil, to prevent insect attack.

Coconut sampling

Harvests were made at 15, 45, 60, 90, 180, 270 and 366 days after treatment. For each variety, 8 coconuts

of 7 months were harvested on 8 trees randomly selected among the 15 trees of treatment. Nuts of each treatment were weighed and shelled. Thereafter, a sample of 125 g was taken from each coconut to form a homogeneous composite sample of 1 Kg. The composite sample was stored in the freezer and later used to measure their phenolic compounds content.

Extraction of phenols

The protocol described by Diabaté*et al.* (2009) was adapted to our plant material and used for this extraction. After homogenization of the composite husk sample, 10 g were taken, weighed and then crushed in 80 ml of absolute ethanol - water mixture (70/30; v / v). This mixture was boiled under vacuum for 30 min. After cooling, the liquid obtained was filtered and dried in a rotary evaporator at 40 °C for 1H. The residue was taken up in 8 ml of absolute ethanol. The extract obtained was stored in a freezer at 20 °C before assays.

Quantification of phenolic compounds

The assay of total polyphenols in the coconut husks was performed using the method of Marigo (1973) with the Folin-Ciocalteu reagent. This method measures the redox potential of phenols. Indeed, in basic medium, the Folin-Ciocalteu reagent oxidizes the oxidizable groups of polyphenolic compounds present in the sample. The reduction products (metallic oxides) of bluecolor, have a maximal absorption whose intensity is proportional to the amount of polyphenols present in the sample (Anonymous, 2010).

The obtained contents were calculated by taking the chlorogenic acid as reference, and expressed in mg of chlorogenic acid per gram of fresh weight. The reading of the optical densities (OD) was performed at 765 nm with the spectrophotometer Secoman S250 (Secoman, France).

Data analysis

The statistical analysis was conducted with Statistica 7.1 software. The data were submitted to a three way analysis of variance (ANOVA) to estimate the significance of husks' polyphenols content. The Newman-Keuls test at 5% probability level was used to separate the different means.

Results

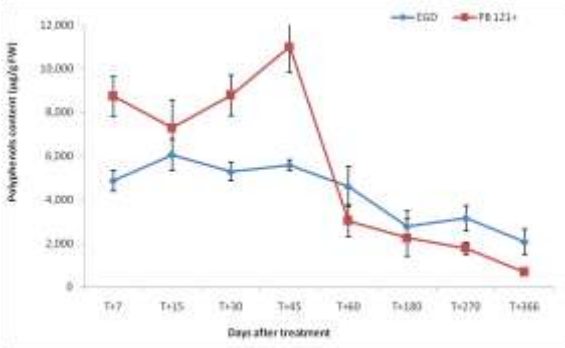
Husk's phenolic compound content

Coconut variety has a significant effect on coconut husk phenolic compound content ($p < 0.001$). The variety PB 121⁺ had the highest phenolic content estimated to 5445.95 µg/g of fresh weight (FW) while the phenolic content of NVE variety was 4298.29 µg/g FW (Table 1). PB 121⁺phenolic content varied from 694 to 11001.83 µg/g FW while the one EGD ranged from 2070.75 to 6049.75 µg/g FW. After 60 days of phosphorus acid application, it an overall decrease of phenolic compound production was observed (Fig. 1).

Table 1. Levels of total phenolics (µg/g FW) based on coconut varieties and phosphorous acid treatment.

Treatments	Varieties	
	EGD	**PB 121⁺**
To (Control)	4126,50b	6433,71d
TA (2.8 g of phosphorous acid)	3918,25a	5754,46c
TB (5.6 g of phosphorous acid)	4309,92c	4594,58a
TC (11.2 g of phosphorous acid)	4838,50d	5001,04b
P	<0,001	<0,001
Means	4298,29A	5445,95B

Means with same letter are not statistically different according to Newman-Keuls test at 5% probability level.



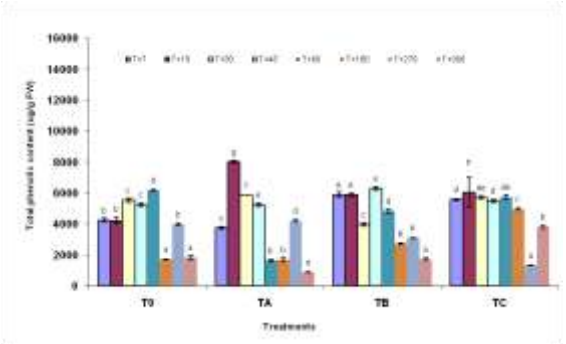
EGD : Equatorial Green Dwarf; **PB 121⁺** : Improved hybrid PB 121⁺.

Fig. 1. Evolution of total phenolics production of EGD and PB 121⁺ varieties according the time.

Change in phenolic compounds production based on phosphorous acid doses and coconut varieties

Coconut variety PB 121+ total phenolic content ranged from 4594.58 to 6,433.71 71 µg/g FW while those determined in the EGD variety varied between 3,918.25 and 4838.5 71 µg/g FW (Table 1).

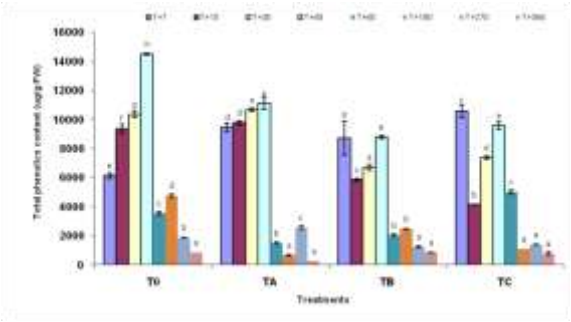
Inthe untreated (To) EGDvariety, the amounts of polyphenolic compounds ofcoconuts husk varied progressivelyfrom 1723 to 6198µg/g FW.TA treatment coconuts trees had their phenolic compound varied between 887 and 8044 µg/g FW. The ranges of total phenolic were between 1728 and 6312 µg/g FW for TB Treatment and between 1331 and 6049 µg/g FW for TC treatment (Fig. 2). Differences were highly significant among the phenolic amountsof the treatments produced during different periods.



To (Control); TA (2.8 g of phosphorous acid); TB (5.6 g of phosphorous acid); TC (11.2 g of phosphorous acid).

Fig. 2. Total phenolic content in the coconut husk of EGD variety as influenced by treatments along time.

For the hybrid PB 121+, levels of total polyphenolic compounds determined in To control trees treated varied between 878 and 14503 µg/g FW during the experiment. For TA treated coconuts trees, the amounts of total phenols ranged between 261 and 11101 µg/g FW. The amounts obtained from TB treated coconuts varied between 862 and 8799 µg/g FW. For TC treated coconut trees, total polyphenols were between 775 and 10580 µg/g FW. The amounts of polyphenols produced differed between the periods of sampling (Fig. 3).



To (Control); TA (2.8 g of phosphorous acid); TB (5.6 g of phosphorous acid); TC (11.2 g of phosphorous acid).

Fig. 3. Total phenolic content in the coconut husk of PB 121+ variety as influenced by treatments along time.

Discussion

Coconuts husk of the two varieties EGD and PB 121+ accumulated total polyphenols. On average, in all treatments, the presence of these compounds was higher in the variety PB 121+, than in EGD variety. This difference could be related to the difference in sensitivity of these two varieties. Indeed, Bourdeix *et al.* (2005) showed that the variety PB 121+ is more tolerant to *Phytophthora* attacks because of having inherited resistance genes from his parents contrary to EGD.

Results of phenolic compounds production by these two varieties are consistent with those obtained by Rubio-Covarrubias *et al.* (2006) and Korgan *et al.* (2011), who have shown on different potato cultivars *Solanumtarijense* that the amount of phenolic compounds produced largely depends on their level of resistance to *Phytophthorainfestans*.

Moreover, nuts husk used to determine total polyphenol contents were from trees treated or not with phosphorous acid. These coconuts have not been inoculated with a strain of *Phytophthorakatsurae*. The coconuts husks form untreated control trees of variety PB 121+ had total polyphenols values higher than those of PB 121+treated plants. In contrary, coconuts husk from untreated control trees of EGD variety accumulated lower total polyphenols than

those of the majority of coconut husk fromtreated plants.

Similarly, the results showed that in the absence of *Phytophthora* attack, phosphorous acid does not disturb the metabolism of the tolerant variety PB 121+ while it slightly modified the one of the susceptible variety EGD by increasing the quantities of polyphenols produced. These results are contrary to those obtained by Trique *et al.* (1981) who found no disturbance of the metabolism of tomatoes (resistant or sensitive) after treatment with tris-o-ethyl phosphonate aluminum (TEPA) which has phosphorous acid as active metabolite. This difference could be explained by the plant material used or by the type of fungicide applied due to the purity of phosphorous acid applied. These first results obtained in this work could be confirmed on other sensitiveor resistant coconut varieties to *Phytophthora*disease.

Nevertheless, the total polyphenol contents varied depending on the treatments and periods in each coconut variety. These were more important at the beginning of the experiments and decreased along time after 60 days. This fact could involve the presence of polyphenols in the total transitional substances. In fact, these substances are important in the plant defense mechanism, andplant cells toxins are degraded by enzymes such as extracellular peroxidases through oxidation (Traore *et al.*, 2005)

Conclusion

Total polyphenols produced in the EGD variety nut husk were lower than those produced by the variety PB 121+. The levels of polyphenols among coconut treated with phosphorous acid were lower than the untreated coconuts of variety PB 121+. Those determined in coconut from treated nuts of NVE variety were higher compared to values obtained in coconuts from untreated trees of the same variety. However, the amounts obtained from these two coconut varieties were of treatment and period-dependant. The determination of total polyphenols in both coconut varieties helped to highlight resistance traits in PB 121+ compared to the EGD. These results confirmed in coconut, the importance of biochemical indicators such as total polyphenols in determining varietal tolerance sources.

To clarify the role of phenolic compounds in the protection of the coconut against *Phytophthora* diseases, it is necessary to determine the different family of polyphenols comprised in the phenolic compounds involve in coconut defense reactions.

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