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Efficacy of the Biopesticide NECO in the control of *Ralstonia solancearum*, causal agent of tomato bacterial wilt in Côte d'Ivoire

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Abstract

The tomato crop is confronted to numerous soilborne pathogens, including *Ralstonia solanacearum*, which considerably limits its production. In order to control this bacterium, a biological control approach has been considered by evaluating the efficacy of the NECO biopesticide against this bacteriosis. *In vitro* confrontations were carried out using a range of five concentrations of the biopesticide. *In vivo*, NECO solutions of 5 and 10 mL/L were incorporated into soil previously infested with *R. solanacearum* before transplanting tomato plants. Zones of bacterial growth inhibition were observed after the application of the NECO biopesticide. Results showed that the 20 mL/L concentration resulted in a higher inhibition rate. The Biopesticide at the 10 mL/L concentration significantly reduced the incidence of bacterial wilt (54.05%) under in vivo conditions. The NECO biopesticide could be used as a control agent for *Ralstonia solanacearum*.

Keywords: Bacterial wilt, *Ralstonia solanacearum*, Biopesticide, tomato, Côte d'Ivoire

Subject Classification: Crop protection

Introduction

Market gardening plays an important role in the agricultural sector and is considered a food sovereignty activity (FAO, 2012). Market gardening plays an important role in most nutrition and poverty alleviation programmes and contributes significantly to improving family incomes (James, 2010). In Côte d'Ivoire, these crops, especially tomatoes (*Lycopersicon esculentum*), are grown in all areas of the country. It provides producers with a substantial income and constitutes an income-generating activity enabling them to meet some vital needs (Mondjedji *et al.*, 2015).

However, tomato cultivation faced to many biotic and abiotic constraints that affect yields and post-harvest operations. Among the biotic constraints, bacterial wilt caused by *Ralstonia solanacearum* causes considerable damage, and is listed as one of the major phytosanitary problems in the world (Mansfield *et al.*, 2012). Bacterial wilt caused by *Ralstonia solanacearum* occurs worldwide, mainly in tropical and subtropical areas (Parkinson *et al.*, 2013).

In Côte d'Ivoire, it was first observed in 1984 in eggplants plots in Adiopodoumé (Declert, 1987) and is now found in almost all production areas with a harmfulness that has led to the abandonment of some plots. In addition, the work of N'guessan *et al*. (2012) has provided information on the existing genetic variability of the strains. Different strategies involving prophylactic measures are used to control this bacterial disease. However, genetic control is considered to be the most effective and promising control strategy (Hayward, 1991). However, the bypass of resistance by some strains of the bacterium, because of its high phenotypic and genotypic variability and its high genomic plasticity, limits the effectiveness of this control method.

Therefore, the search for alternative methods for effective and healthy control is essential. Biological control could be an alternative, because it has a much more targeted impact and a very low persistence in the environment (Amoatey and Acqual, 2010). One of its approaches is the use of plant extracts and essential oils capable of preventing or limiting the proliferation of the parasite. Various studies have demonstrated the antibacterial and antifungal activity of certain plant extracts (Oxenham *et al*., 2005). Concerning bacterial wilt, various works have shown that aqueous extracts and essential oils from certain plant species of Indian wood (*Pimenta racemosa*), spring onion (*Allium fistilosum*), *Cymbopogon citratus*, *Eucalyptus* significantly reduce the incidence of the disease (Deberdt *et al*., 2012; Paqueroles, 2012, Paret *et al*. 2010). In this context, plant extracts could be used to reduce the incidence of this bacterial disease.

The objective of this work was to study the effect of the Biopesticide NECO on the incidence of bacterial wilt caused by *Ralstonia solanacearum*.

Materials and Methods

Plant material

The plant material consisted of two varieties of tomato. These are the Petomech and Lindo varieties. The seeds were obtained from the seed company Semivoire Abidjan (Côte d'Ivoire).

Bacterial strains

Two strains of *Ralstonia solanacearum* RUN 1743 (phylotype I; sequevar 31) and RUN 1744 (phylotype I; sequevar 31) were used. They originate respectively from Daloa and Man localities (N'Guessan *et al*., 2012).

Essential oils

The Biopesticide NECO based on *Ocimum gratissimum* essential oil was used in the trial to control this bacterial disease. The Biopesticide was supplied by the Industrial Research Unit (URI) on essential oils of the Scientific and Innovation Pole of Felix Houphouët Boigny University of Abidjan.

Culture and purification of bacteria

The bacterial strains in lyophilized form, were suspended in nutrient broth for 48 hours in an oven at 28°C. The bacterial colonies in the nutrient broth were then inoculated onto CPG medium. After seeding, the Petri dishes were incubated at 28°C. After three days of incubation, *Ralstonia solanacearum* colonies were purified on new culture media to obtain typical *R. solanacearum* colonies.

In vitro evaluation of the antibacterial activity of the Biopesticide NECO

Five concentrations (1, 3, 5, 10, 20 mL/L) of NECO were tested under *in vitro* conditions. The method used was the diffusion test. Bacterial inoculum at the concentration of 10^8 CFU/mL was prepared from the 24-hour colonies of *R. solanacearum* strains RUN 1743 and RUN 1744. A volume of 1 mL of the inoculum was distributed and homogenized in Petri dishes containing the CPG medium. Petri dishes were left half-open in the flame for brief drying. After drying, three to four wells were made per Petri dish in which a volume of 40 µL of each NECO solution was delicately deposited depending on the concentrations. The control was carried out with sterilized distilled water. After diffusion of the product, the Petri dishes were incubated in the oven. After 24 hours, the bacteria growth inhibition diameters were measured using a ruler on two perpendicular axes drawn on the back of each Petri dish through the center of each well.

In vivo evaluation of the Biopesticide NECO against bacterial wilt

Substrate treatment and transplanting of plants

Soil from the Songon (Dabou) vegetable production area was sterilized and then inoculated with RUN 1743 strain. This inoculum was calibrated at an optical density (OD) of 0.2 at a wave length of 600 nm. 500 mL pots were filled with 500g of sterilized soil. The soil in each pot was then artificially infested by adding 20 mL of the inoculum. A curative treatment was carried out by incorporating 10 mL of NECO biopesticide solution at concentrations of 5 and 10 mL/L. Positive controls consisted of inoculated, non-NECO-treated substrates while negative controls consisted of non-NECO-treated, non-inoculated substrates. A 3-week-old tomato plant was transplanted into each pot 5 hours after treatment of the soil with NECO. The pots containing the plants were placed in a greenhouse. The temperature in the greenhouse varied between 26.7 and 30°C. Regular watering was carried out to maintain moisture conditions favorable for disease expression. Ten plants per treatment and per variety were used, arranged in 4 blocks. Each block contains one treatment. A total of 4 treatments were performed in this study.

-Uninoculated, unamended sterilized soil with NECO (Negative control noted T0)

-Inoculated sterilized soil and unamended with NECO (Positive control noted T0_in).

- Sterilized soil inoculated and amended to a concentration of 5mL/L of NECO (noted C5).

- Sterilized soil inoculated and amended to a concentration of 10 mL/L of NECO (noted C10).

Monitoring of the disease

Symptoms were monitored from the third day after inoculation for 28 days. Symptoms were assessed every two days according to the scale adopted by (Coupat-Goutaland *et al.*, 2011).

Various parameters were calculated 28 days after observation using the rating scales. These were pathological parameters including wilting index (WI), colonization index (CI), and disease reduction rate (DR).

- The Wilt Index (WI) (Coupat-Goutaland *et al.*, 2011) reflects the incidence of disease by taking into account scores 3 and 4.

$$WI\ (\%) = \frac{N3 + N4}{N} X\ 100$$

With N3: Number of plants with note 3; N4: Number of plants with note 4; N: Total number of plants observed.

- The reduction of Disease rate (RDR) reflects the effectiveness of the treatments in controlling bacterial wilt. It is calculated from the disease index (IM)

$$RDR\ (\%) = \frac{IMc\ - IMt}{IMc} X\ 100$$

IMc: disease index of control plants; IMt: disease index of treated and inoculated plants.

Assessment of latent infections on asymptomatic plants

At the end of the trial, isolation on tomato stems was carried out on asymptomatic plants in order to verify the presence or absence of *Ralstonia solanacearum*. Sections of 2 to 3 cm of the stem of each plant were made at the crown level followed by disinfection with alcohol. These sections were transferred to 5 mL of distilled water and left for 2 h at room temperature to promote the release of bacterial colonies in the distilled water. 50 µL of each extract was spread on SMSA medium. Petri dishes were then incubated at 28°C for 3-4 days. Asymptomatic plants were noted to be positive for latent infection when characteristic colonies of *R. solanacaerum* were observed. Data obtained from latent infections were used to calculate the Colonization Index (CI)

$$CI = WI + (NS\ X\ RS)$$

with WI was wilting index, NS was asymptomatic plants rate and RS was infected asymptomatic plants rate

Statistical analysis

These data were analyzed using Statistica version 7.1 software. An Analysis of Variance (ANOVA I) was performed and in case of significant difference between the means, the separation of the means was done by the Newman-Keuls test at the 5% threshold.

RESULTS

Effects of the Biopesticide NECO on the *in vitro* growth of bacterial strains

Zones of inhibition of bacterial growth were observed after the application of NECO at different concentrations. Statistical analysis showed a significant difference between the different diameters of inhibition, however there was no significant difference between the 10 and 20 mL/L concentrations (**Fig.1**). The largest diameters of inhibition were observed at the 20 mL/L concentration with 1.80 cm and 1.90 cm for strains RUN 1743 and RUN 1744, respectively.

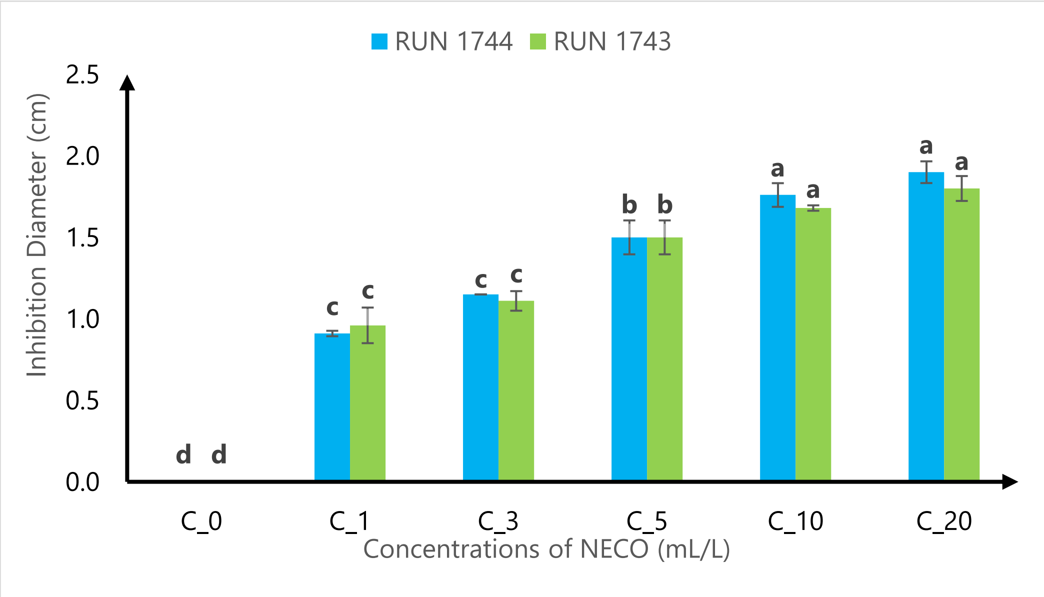

Figure 1: Effect of the Biopesticide NECO on the in vitro growth of *R. solanacearum* strains

Histograms affected by the same letter are not significantly different at the 5% level (Newman – Keuls test)

Incidence of the Biopesticide NECO application on *in vivo* Development of Bacterial Wilt

Effect of NECO on wilting index

Analysis of the results presented in **Fig. 2** showed that there is a significant difference at the 5% threshold between wilt index values. Control tomato plants showed the highest wilt indices (90%) compared to plants planted on treated soils. Wilt indices were lower at the 10 mL/L concentration. However, Lindo had the lowest wilting index (10%) at this concentration compared to Petomech (IF = 20%). The highest wilting indices were recorded with the Petomech variety for the two concentrations tested, 40% and 20%, respectively.

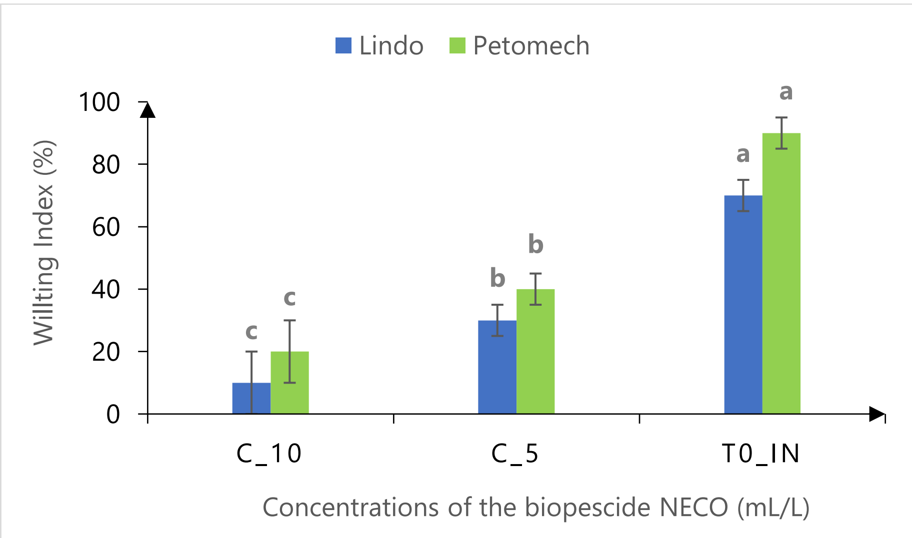

Figure 2: Effect different concentrations of NECO on tomato wilting index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

T0_in: Inoculated sterilized soil and unamended with NECO, C5 Sterilized soil inoculated and amended to a concentration of 5mL/L of NECO, C10- Sterilized soil inoculated and amended to a concentration of 10 mL/L of NECO

Histograms affected by the same letter are not significantly different at the 5% level (Newman – Keuls test)

Effect of NECO on bacterium colonization index in tomato plants

Results showed significative differences between the concentrations of the Biopesticide. The Index Colonization were higher for Lindo plants at the concentrations of 10 mL/L (100%) against 50% for the plant of the Petomech variety. Also , this index was low at the concentration of 5 mL/L for Lindo (60%) compared to Petomech (**Fig. 3**).

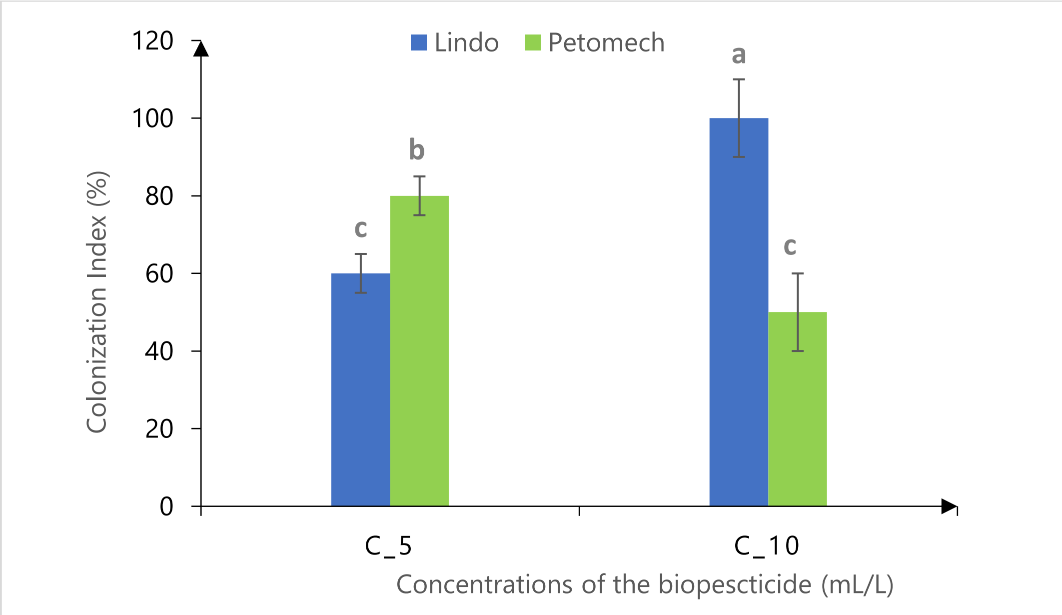

Figure 3: Effect of NECO on tomato colonization index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

Histograms affected by the same letter are not significantly different at the 5% level (Newman – Keuls test)

Effect of the Biopesticide NECO on wilt reduction rate

Significant differences at the 5% threshold were recorded between the percentage wilt reduction for the two concentrations C_5 and C_10 (**Fig. 4)**. However, at each concentration, there was no significant difference between the two varieties (Lindo and Petomech). Disease reduction is relatively greater at the C_10 concentration than at the C_5 concentration. The highest percentage of reduction was observed at C_10 concentration with the variety Petomech (54.05%) while the lowest percentage was obtained at C_5 concentration with the variety Lindo (28.75%).

DISCUSSION

The *in vitro* evaluation of the Biopesticide NECO showed a very good ability to reduce bacterial growth. The results showed variable inhibition zones depending on the five concentrations. The 20 mL/L concentration showed greater inhibition of bacterial growth. These results corroborate those of some authors who have confirmed the *in vitro* efficacy of NECO on *Xanthomonas* sp. responsible for bacterial diseases of cashew nuts and cassava (Koné, 2018; Affery *et al.*, 2018, Soro *et al.*, 2011). This antibacterial efficacy of NECO evaluated *in vitro* conditions is also confirmed by Silué et *al*. (2018) on *Colletotrichum gloeosporioides* and *Pestalotia heteronis*, fungi responsible for cashew nut leaf diseases.

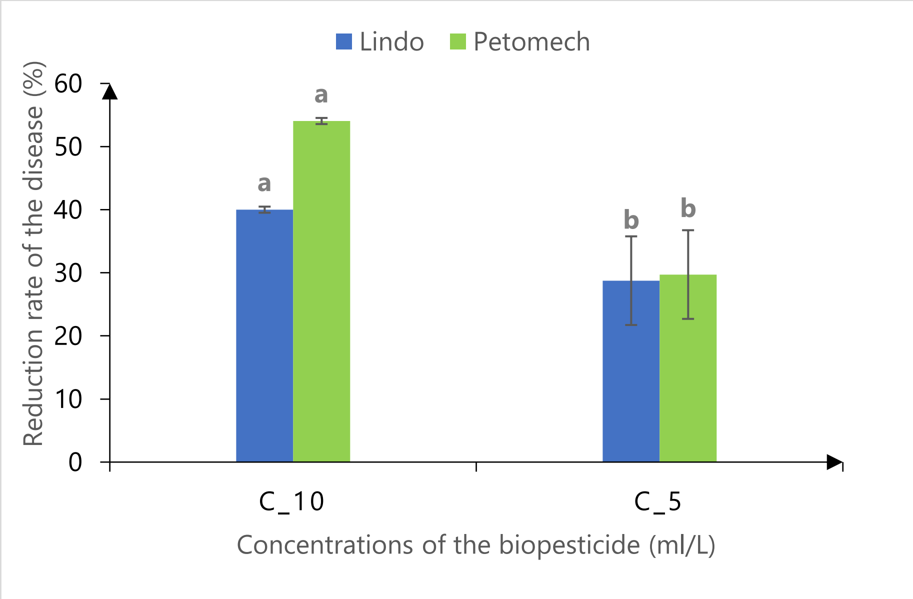

Figure 4: Effect of NECO on tomato bacterial wilt reduction rate after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

Histograms affected by the same letter are not significantly different at the 5% level (Newman – Keuls test)

This antibacterial effect of the Biopesticide was also confirmed in vivo. In this study, the behaviour of tomato plants was observed in a soil artificially infested with *R. solanacearum* followed by treatment with NECO. The effect is even more pronounced when the concentration of the Biopesticide is high. Disease incidence in treated plants is relatively lower than in controls. The 10 mL/L concentration was most effective in reducing the disease by up to 54.05% in the susceptible Petomech variety.

Similar results were obtained in an in vivo study by Silué *et al*. (2018) who showed that the use of NECO against *Colletotrichum gloeosporioides*, the agent responsible for cashew nut leaf disease at concentrations of 1000 ppm reduced the severity of the disease by more than 50%. Work by Kassi *et al*. (2014) showed that NECO applications at 5mL/L reduced the impact of black banana cercosporiosis. The observed antibacterial activity of the Biopesticide NECO could be explained by the existence of active ingredients with very strong antibacterial properties that would act directly on the crop pathogens and on the propagation organs (Camara *et al*., 2007). This property would be due to the action of phenolic compounds such as thymol which is the major component of the essential oil of *Ocimum gratissimum* from which NECO was formulated. Thymol is known to be toxic to microorganisms and is thought to target the cytoplasmic membrane and wall of these microorganisms (Kassi *et al*., 2014). Indeed, much work has highlighted the efficacy of phenolic compounds, particularly that of thymol which has a very broad spectrum of antimicrobial action (Ajouri *et al*., 2008; Bounatirou, 2007). Also, camphor and 1,8- cineole, two of the constituents of NECO, are believed to inhibit the germination of propagating or infecting organs and the growth of pathogens from these organs.

Conclusions

This study demonstrated the efficacy of the Biopesticide NECO against *Ralstonia solanacearum*, a bacterial wilt agent. *In vitro*, the 20 mL/L concentration provided the highest rate of inhibition. *In vivo*, the 10 mL/L concentration resulted in a reduction in disease incidence of approximately 54.5%. This work can be considered as an ecological approach and an alternative to chemical control of bacterial wilt caused by *R. solanacearum* in tomato.

Conflicts of Interest

There are no conflicts of interest.

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