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Research article

Field efficacy of aqueous extracts of *Artemisia annua, Commelina benghalensis* and *Euphorbia hirta* on rice growth, yield and brown spot disease incidence

Joseph Fovo Djeugap[a,*], Biamen Mbatkam[a], Arlette Meli Sonkoue[b,†], Sylvere Lontsi Dida[c,†], Joseph Hubert Yamdeu Galani[d,†], Claudette Mbi[a], Melanie Lapa[a], Kenneth Yongabi Anchang[e,†]

[a] *Phytopathology and Agricultural Zoology Research Unit, Department of Agriculture, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Dschang 222, Cameroon*

[b] *Applied organic chemistry Research Unit, Department of Chemistry, Faculty of Science, University of Dschang, Dschang 76, Cameroon*

[c] *Phytopathology and Plant Protection Research Unit, Department of Plant Biology, University of Yaoundé, Yaoundé 812, Cameroon*

[d] *Section of Natural and Applied Sciences, School of Psychology and Life Sciences, Faculty of Science, Engineering and Social Sciences, Canterbury Christ Church University, Canterbury, Kent CT1 1QU, UK*

[e] *Department of Public Heath, Faculty of Health Sciences, Imo State University, Owerri, PMB 2000 Nigeria*

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Abstract

Importance of the work: Brown leaf spot disease is one of the major causes of yield losses in rice production in Cameroon.

Objectives: To evaluate the bioactivity of aqueous extracts of three Cameroonian medicinal plants on rice growth, yield and brown spot disease incidence.

Materials & Methods: Plant extracts were tested at two doses (1 kg/15 L and 2 kg/15 L sprayed), while water and Mancozeb were used as negative and positive controls, respectively, in a split plot design made up of two factors (variety and plant extract). The varieties were NERICA 8, NERICA L56, Long grain red rice and Toukbem.

Results: NERICAs 8 and L56 (exotic) and, Toukbem (local) varieties had the highest number of leaves and tillers while Toukbem had the highest plants and leaf area at 9 wk after transplanting. The numbers of leaves (31.3) and tillers (9.7) were significantly ($p < 0.05$) higher in plants treated with *Euphorbia hirta* extract at 67 g/L compared to *Artemesia annua* and *Commelina benghalensis* extracts. Brown spot severity was lower with *E. hirta* at 134 g/L (30%) and not significantly different to Mancozeb (23.6%). The NERICA L56 × *E. hirta* interaction at 134 g/L had the highest yield (5.8 t/ha) compared to the NERICA L56 × Mancozeb (6.1 t/ha) interaction. Chemical analysis of the *E. hirta* aqueous extract showed the presence of known bioactive compounds.

Main finding: Field application of aqueous extract of *E. hirta* at 134 g/L could be used as a bioagent for rice brown spot management. The formulation of a natural product with this extract is necessary for large-scale field applications.

† Equal contribution.

* Corresponding author.
E-mail address: jdjeugapfovo@yahoo.fr (J.F. Djeugap)

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Introduction

Rice (*Oryza sativa* L.) is a staple food item for more than 3.5 billion people worldwide being cultivated over an area of about 160.68 million ha, with an annual production of about 650.19 million t (Tomar et al., 2018). About 90.3% of the world's rice production comes from Asia while 5.2% and 3.8% comes from America and Africa, respectively with Cameroon having a low rate of production which keeps on decreasing from 359,320 t in 2016 to 328,503 t in 2020 (Food and Agriculture Organization of the United Nations, 2020), despite the attention directed toward it by the Ministry of Agriculture and Rural Development. For decades, the yields have remained low (1–6 t/ha) according to Ministry of Agriculture and Rural Development (2009) and this has been attributed to neglect of the sector by the government, cultural techniques, and pests and diseases (Djomo et al., 2017). In Cameroon, the most severe rice disease is brown spot caused by *Bipolaris oryzae* which is responsible for yield reductions in the range 15–52% (Kuate et al., 2021). Disease management through the use of chemical has been reported to be effective for most cereal crops (Kongcharoen et al., 2020). Fungicides have played an important role in increasing crop production over the past decades, although crop production with fungicides has not been sustainable due to its high cost, residual toxicity, development of resistance, environmental pollution and health hazards to humans and animals (Galani et al., 2020). Thus, the use of plant extracts for the control of rice fungal leaf-borne diseases can lead to the sustainable use of plants and may become a practice with economic and environmental benefits. Notably, Cameroon has a rich diversity of plants that contain alkaloids, tannins, quinones, coumarins, phenolic compounds and phytoalexins, which are known for antifungal activity (Galani et al., 2013; Gupta et al., 2018). Furthermore, most of the research work on the bio-efficacy of botanicals against plant diseases has been limited to *in vitro* assays, with a few glasshouse experiments. Therefore, it is important to understand their efficacy under field conditions. The current study was conducted to evaluate the biological activity of three plant extracts on brown spot incidence and the growth and yield of rice in the field. The plant extracts tested were selected based on their efficiency against other plant and human diseases as reported in the literature (Gbekley et al., 2017).

Material and Methods

Seed collection

The seeds of the rice varieties NERICA 8 and NERICA L56 (exotic) and, Long grain red rice and Toukbem (local) were obtained from the Upper Nun Valley Development Authority (UNVDA), in Ngoketujia (North-West region of Cameroon), while the seed samples of the local rice varieties (Long grain red rice and Toukbem) were obtained from farmers in Tonga (West region of Cameroon).

Plant extract preparation and application

Samples of 1 kg and 2 kg of fresh plant material from each of *E. hirta, C. benghalensis* and *A. annua* were collected in Dschang sub-division (5°26'45.3804''N, 10°2'49.758''E at an elevation of 1,327.40 m above mean sea level; amsl). The samples were washed, ground and placed in a clean container. Then, 10 L of the extraction solvent (cold water) was added to the plant material, soaked and kept for 24 hr in a rotary incubator. The solution was filtered after 24 hr of maceration using a mousseline cloth. The quantity of extracts obtained was completed with water to make up to the final volume of the tank of the Knapsack sprayer used (15 L). Each plant extract was applied at the rate of 23.25 kg/ha and 46.5 kg/ha (which represented 1kg of extract/15L and 2 kg of extract/15L, respectively, of spray). This was equivalent to concentrations 67 g/L and 134 g/L, respectively. Aqueous plant extracts were used directly after 24 hr of maceration.

Phytochemical screening and determination of contents of total phenols, flavonoids and tannins in E. hirta extract

The identification of phytochemical compounds was carried out only with the *E. hirta* extract, which showed a high antifungal potential. This screening was performed based on qualitative staining methods, according to Harbone (1973). The total phenols content was determined by the method described by Ramde-Tiendrebeogo et al. (2012). The reagent is a mixture of phosphotungstic acid and phosphomolybdic acid. It is reduced, during the oxidation of the phenols, to a mixture of blue oxides of tungsten and molybdenum. These blue pigments have a maximum absorption that varies according to the qualitative or quantitative composition, or both, of the phenolic mixtures, in addition to the pH of the solutions,

usually obtained by adding sodium carbonate. The results were expressed as the milligram equivalent of gallic acid per gram of powder.

The total flavonoid content was determined using the aluminum chloride colorimetric method (Chang et al., 2002). A volume of 100 µL of extracts (2 mg/mL) was mixed with 50 µL of aluminum chloride (1.2%); then, 50 µL of potassium acetate (120 mM) was added. The total flavonoid content was calculated using the quercetin calibration curve (quercetin concentration was in the range 0.015–2 mg/mL) and the results were expressed as the milligram equivalent of quercetin per gram of powder.

The total tannin content was determined based on the Folin-Ciocalteu method, as described by Govindappa et al. (2011). Here, the reaction mixture consisted of 100 µL of extract, 500 µL of Folin-Ciocalteu reagent (diluted 10-fold in water), 1,000 µL of 35% sodium carbonate solution and 8.4 mL of distilled water. The mixture was stirred and incubated at room temperature for 30 min; then the absorbance was measured using a spectrophotometer at 700 nm. The results were expressed as the milligram equivalent of tannic acid per gram of powder.

Field experimentation

The study was carried out in Tonga sub-division (4°58'0.01"N, 10°41'60.00"E at an elevation of 819.85 m amsl) from March to August 2021. The experimental design was a complete randomized block design with a split plot arrangement. For each block, the principal factor was the rice variety (NERICA 8, NERICA L56, long grain red rice or Toukbem) and the second factor was plant extracts. The seeds were put in silver pots and watered during four days for pre-germination. The number of repetition was five and the experimental plot occupied a total surface area of 430 m^2. The pre-germinated seeds were sowed in a seedbed (6 m × 2 m) with a spacing of 0.5 m between the four rice varieties. Twenty-six seedlings (about 4–6 leaves) were transplanted to the experimental field at 25 cm × 25 cm spacing. Thinning was done 10 d after transplanting (DAT). Plants were thinned to two individuals per stand. Fertilizer was applied three times to improve the uptake mineral nutrients needed for the optimal growth of the plants, as recommended by Ministry of Agriculture and Rural Development (2009). In total, 200 kg/ha of NPK (20-10-10), 50 kg/ha of urea and 50 kg/ha of urea was applied at 14 DAT, 46 DAT and 73 DAT, respectively (Goufo, 2008; Ministry of Agriculture and Rural Development, 2009). Weeding was done manually once a month using a hand hoe. The varieties used were reported to be susceptible or tolerant to diseases in the agroecological zones of Cameroon where they are cultivated. Natural infection was allowed to develop in the field as occurs in the rice farmer plantations. However, after the establishment of natural infection, samples showing brown spot symptoms were collected and transported to the laboratory for pathogen isolation and identification. This confirmed that the natural infection observed was due to *Bipolaris oryzae*. Each plant extract (*A. annua, C. benghalensis* and *E. hirta*) was tested at the rate of 1kg/15L (67 g/L) and 2 kg/15L (134 g/L) and applied on the aerial parts of the rice crop using a 15 L Knapsack sprayer. Mancozeb at the recommended dose (3.33 g/L) and water were used as positive and negative controls, respectively. The extracts and Mancozeb were applied at a frequency of 10 d, as recommended for this homologated chemical (8–14 d). Imidacloprid (a systemic insecticide) was used to control insects in the field while bird management at the time of panicle appearance was by a combination of three methods (the use of cassette tape, the scarecrow method, and the use of nets). Weed management was done manually. All treatments were repeated five times. Rainfall and temperatures were recorded at the meteorological station of the National Institute of Research in Agriculture located 1 km from the experimental site.

Data collection

The collection of growth variables started 21 DAT before the onset of flowering (Djomo et al., 2017) on five plants per experimental unit. The growth variables measured were: plant height (in centimeters), leaf area (in square centimeters) based on leaf length and width (in centimeters) and the numbers of tillers and leaves. The incidence and severity of brown spot disease were measured weekly. These disease variables were calculated using the calculation Incidence = 100 × (Number of diseased plants × Total number of plants), according to Chowdhury et al. (2013). Disease severity was measured on a 0–9 scale based on standard evaluation (International Rice Research Institute, 2009), where 0 = no incidence; 1 = less than 1% leaf area affected; 2 = 1–3% leaf area affected; 3 = 4–5% leaf area affected; 4 = 6–10% leaf area affected; 5 = 11–15% leaf area affected; 6 = 16–25% leaf area affected; 7 = 26–50 leaf area affected; 8 = 51–75% leaf area affected and 9 = 76–100% leaf area affected. The severity was assessed as the percentage of the infected area of the plant over the total area considered. The number of panicles produced and the yield were estimated at harvest.

Statistical analysis

Data were entered into the Microsoft Office Excel software (version 2013; Microsoft Corp.; Redmond, WA, USA) and histograms were plotted using the same software. Then, the data were subjected to a two-way analysis of variance test using the R software package, version 4.0.1 (R Core Team, 2021). Data as percentages were submitted to angular transformation (or arcsine square-root transformation) prior to analysis of variance. The data tested followed a normal distribution (Shapiro-Wilk test) and were also homogeneous (Levene test). For each variable having a significant effect, a mean separation was carried out using a least significant difference test. The tests were considered significant at $p < 0.05$.

Results and discussion

Effect of variety on growth, brown spot and yield variables of rice

The number of leaves of rice plants was significantly influenced by the varieties used. NERICA 8 produced the highest number of leaves (31.9) which was significantly different from Long grain red rice (LGRR) while the number of leaves of Nerica 8 was not significantly different from Nerica L56 and Toukbem at 9 wk after transplanting (WAT), as shown in Table 1. The number of tillers, plant height and leaf area of rice plants were also influenced by the variety: NERICA L56 had the highest tiller number (9.7) and LGRR had the lowest number (6.1). Toukbem had the greatest plant height (22.7 cm) and leaf area (28.1 cm^2), while the lowest plant height (17.4 cm) and leaf area (13.4 cm^2) were recorded for NERICA 8 at 9 WAT. The effect of variety was not significant on brown spot incidence, irrespective of the collection date. However, NERICA L56 had the lowest disease severity (26.8%) at 90 DAT (Table 2). The disease severity of LGRR (47.5%) was significantly higher than for Nerica L56 at 90 DAT, while LGRR brown spot severity was not significantly different from Nerica 8 and Toukbem. Disease severity of NERICA L56 which was least susceptible (38%), was not significantly different to Nerica 8 severity (45.3%), as shown in Table 2. NERICA L56 produced the highest number of panicles (4.14) and in addition had the greatest yield (5.2 t/ha) which was statistically different from the other varieties, while NERICA 8 recorded the least yield (2.1 t/ha), as shown in Table 3. The plant height was lower than reported by Faruq et al. (2015) when rice seeds were dipped in neem leaf and allamanda leaf extract during 15 min before sowing. This could have been due to the rice variety used or because of the different techniques of application of the plant extracts; they used the hybrid rice variety Taj-1 of the line GRA-2/06 and plant extracts were applied on seeds in their experiment. Plant growth performance

Table 3 Effects of rice varieties on number of panicles and yield

Variety	Number of panicles		Yield
	100 DAT	110 DAT	(t/ha)
Nerica 8	0.38±0.12^b	1.65±0.31^c	2.1±0.3^c
Long grain red rice (local)	1.06±0.16^a	3.55±0.33^{ab}	4.3±0.5^b
Nerica L56	1.36±0.14^a	4.14±0.37^a	5.2±0.7^a
Toukbem (local)	0.57±0.13^b	2.84±0.35^b	2.5±0.4^c

DAT = days after transplanting

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different

Table 1 Effects of rice varieties on growth variables at 9 wk after transplanting

Variety	Number of leaves	Number of tillers	Plant height (cm)	Leaf area (cm^2)
Nerica 8	31.9±7.23^a	9.5±3.68^a	17.4±3.46^c	13.4±4.19^d
Long grain red rice	22.1±10.02^b	6.1±1.58^b	22.5±3.85^{ab}	27.1±9.92^b
Nerica L56	30.6±4.52^a	9.7±2.58^a	18.8±2.56^{bc}	16.6±5.12^c
Toukbem	29.3±6.03^a	8.5±1.99^a	22.7±2.82^a	28.1± 9.93^a

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Table 2 Effects of rice varieties on incidence and severity of brown leaf spot

Variety	Incidence (%)		Severity (%)	
	30 DAT	90 DAT	30 DAT	90 DAT
Nerica 8	47.7±10.81^{a*}	58.9±13.9^a	23.2±8.79^a	38±8.47^{ab}
Long grain red rice (local)	45.3±9.21^a	67.1±11.84^a	22.2±7.1^a	47.5±11.67^a
Nerica L56	50.1±13.63^a	70.2±16.01^a	15.6±6.3^b	26.8±8.93^b
Toukbem	47.8±12.53^a	66.7±14.69^a	20.2±9.76^a	45.3±10.11^a

DAT = days after transplanting; LGRR = Long grain red rice

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different

(the number of leaves and tillers and the plant height) corroborated those obtained on NERICA 1 and 5, Yumenohatamochi and Hinohikari (two local Japanese varieties) in a non-irrigation cultivation system (Kikuta et al., 2017). In addition, the two NERICA varieties (exotic) used produced the highest numbers of tillers compared to the local varieties, while the leaf area was greater in the Toukbem variety. This difference may be due to differences in the tillering ability (a genetic trait) of each variety. The growth variables in the current study could have been improved by integrating organic fertilizers in the soil. For example, poultry manure (with a higher nutrient content and rate of mineralization) increased the growth performance of rice plants in at all growing stage (Sims and Wolf, 1994; Muhammed et al., 2003).

Chemical composition of aqueous extracts of E. hirta

The most active plant with high antifungal potential was *E. hirta.* The phytochemical screening of the aqueous extract of this plant showed the presence of secondary metabolites, such as alkaloids, phenols, flavonoids, triterpenoids and tannins. The total phenols, flavonoids, and tannins contents were 66.79 gallic acid equivalent (GAE)/g, 3.91 GAE/g and 44.5 mg GAE/g of extract, respectively, (Table 4). Due to their high contents of various bioactive compounds, plants are the main raw material for the production of valuable and useful bio-products with both growth regulators and antimicrobial potential (Luh et al., 2020; Mkindi et al., 2020; Galani et al., 2013). For example, soybean leaf extracts contain high levels of flavonoids, such as salicylic, 4-hydroxybenzoic, vanillic, 4-hydrooxycinnamic, ferulic, caffeic, gentisic, and quercetin, with high protective properties (Mkindi et al., 2020), while terpenes and flavones may breakdown microbial cell membranes (Urzua et al., 2006). Tannins can inhibit electron transport through membranes and can alter ions (such as iron and copper), thus inhibiting the activity of some enzymes which may be essential for microbial life (Luh et al., 2020).

Bioactivity of plant extracts on rice growth, brown spot and yield variables

The monthly average rainfall during the experimental period (March–August 2021) varied in the range 75–255 mm, with the maximum rainfall in June (which corresponded to 90 d after sowing), while the monthly average temperature was in the range 18–24 °C. The analysis of variance showed that the plant extracts had a significant effect on the growth, brown spot incidence and yield variables. *E. hirta* at 1kg/15L (67 g/L) and Mancozeb produced the highest numbers of leaves (31.3 and 30.5, respectively), while *C. benghalensis* at 1 kg/15 L (67 g/L) and 2 kg/15 L (134 g/L) produced the lowest (26.3 and 27.4, respectively), at 9 WAT. The number of tillers was highest with *Euphorbia* 67 g/L (9.7) which was significantly different from the other plant extracts (Table 5). Mancozeb produced the tallest plants (21.5 cm); followed by Euphorbia at 67 g/L (21.1 cm), while *Euphorbia* at 134 g/L produced the shortest plants (19.3 cm), which was not significantly different from *A. annua* at 67 g/L (19.9 cm) or the control (19.2 cm).

Table 4 Some secondary metabolites and phenolic compounds present in aqueous extract of *E. hirta*

Secondary metabolite or phenolic compound	Euphorbia hirta
Alkaloids	+
Phenols	+
Flavonoids	+
Sterols	-
Triterpenoids	+
Tannins	+
Saponins	-
Anthocyanins	-
Anthraquinones	-
TPC (mg GAE/g of extract)	66.79± 0.15
TFC (mg EQ/g of extract)	3.91± 0.27
TTC (mg TAE/g of extract)	44.5± 0.29

+ = present ; – = absent ; TPC = total phenol content ; TFC = total flavonoid content ; TTC = total tannin content; GAE = gallic acid equivalent; EQ = equivalent of quercitrin; TAE = tannic acid equivalent; Values shown as mean ± SD.

Table 5 Effect of plant extracts application on growth variables of rice at 9 wk after transplanting

Plant extract	Number of leaves	Number of tillers	Plant height (cm)	Leaf area (cm^2)
Mancozeb	30.5 ± 10.03^{a}	8.8 ± 3.46^{b}	21.5 ± 4.15^{a}	21.8 ± 9.58^{a}
A. annua (67 g/L)	27.7 ± 6.77^{b}	8.2 ± 2.06^{b}	19.9 ± 4.31^{cd}	22.1 ± 11.09^{a}
A. annua (134 g/L)	28.5 ± 7.10^{a}	8.4 ± 2.36^{b}	20.6 ± 2.61^{abc}	19.4 ± 9.79^{a}
C. benghalensis (67 g/L)	26.3 ± 6.66^{c}	7.7 ± 2.44^{b}	21.1 ± 3.22^{ab}	22.7 ± 9.92^{a}
C. benghalensis (134 g/L)	27.4 ± 7.77^{c}	8.6 ± 3.30^{b}	20.5 ± 4.47^{bc}	21.6 ± 10.81^{a}
E. hirta (67 g/L)	31.3 ± 10.62^{a}	9.7 ± 3.85^{a}	21.1 ± 4.97^{ab}	21.7 ± 10.24^{a}
E. hirta (134 g/L)	28.5 ± 6.58^{a}	8.3 ± 3.05^{b}	19.3 ± 3.54^{d}	17.6 ± 7.41^{a}
Control (water)	28.2 ± 8.07^{a}	7.9 ± 2.47^{b}	19.2 ± 3.24^{d}	23.5 ± 10.42^{a}

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Table 6 Effect of plant extracts application on rice brown leaf spot incidence and severity

Plant extract	Incidence (%)		Severity (%)	
	60 DAT	90 DAT	60 DAT	90 DAT
Mancozeb	15.2±8.5^{b}*	21.5±5.29^{c}	21.4±10.05^{b}	23.6±6.41^{c}
A. annua (67 g/L)	52.2±7.75^{a}	60.1±8.12^{b}	24.7±13.99^{b}	38.9±7.93^{b}
A. annua (134 g/L)	46.1±14.75^{ab}	57.7±7.31^{b}	21.2±7.82^{b}	36.4±5.28^{b}
C. benghalensis (67 g/L)	49.7±12.64^{ab}	52.2±8.05^{b}	27.0±11.31^{b}	37.5±5.22^{b}
C. benghalensis (134 g/L)	49.3±14.98^{ab}	51.8±7.83^{b}	23.8±8.37^{b}	35.5±7.41^{b}
E. hirta (67 g/L)	43.2±10.37^{ab}	45.2±6.76^{bc}	27.7±11.96^{b}	38.5±8.82^{b}
E. hirta (134 g/L)	16.7±12.64^{ab}	24.3±6.47^{c}	26.1±13.7^{b}	29.7±6.85^{c}
Control (water)	53.1±10.42^{a}	72±10.53^{a}	44.8±10.63^{a}	55.0±10.02^{a}

DAT = days after transplanting

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

The plant extracts had no significant effect on the leaf area (Table 5). The number of leaves in rice plants was related to the number of tillers. Thus, an increase in the number of leaves led to an increase in tiller number and hence high production. Secondly, plant height was influenced by the interaction between variety and treatment. This can be explained by the growth regulators present in the plant extracts that improved the growth variables (Silva et al., 2008; Ijato et al., 2010). Jang and Kuk (2019) reported that plant extracts hade growth promotion effects on various crops, which was corroborated by the current results. The same outcomes resulted with extract from *Tephrosia vogelii* and *Tithonia diversifolia* applied as foliar sprays on beans at a concentration of 10% *weight per volume, which* increased the chlorophyll content, the number of pods per plant bean and the overall seed yield (Mkindi et al., 2020). In corn, a foliar application of 100 mg/L crude extract of *Gleicheni linearis* leaves increased plant height and leaf area (Aulya et al., 2018). In the current study, brown spot incidence and severity were significantly higher in plants treated with water (negative control; 72% and 55%, respectively), at 90 DAT compared to the other treatments. Disease incidence and severity with Mancozeb (21.5% and 23.6%, respectively) were not significantly different from *E. hirta* at 134 g/L (27.3% and 29.7%, respectively) at 90 DAT, while disease incidence and severity with *E. hirta* at 134 g/L were significantly different to the other plant extracts. In addition, plants that received extract of *E. hirta* at 134 g/L had the highest yields (5.8 t/ha) which was not significantly different from Mancozeb (5.6 t/ha), as shown in Table 7. Comparable results were obtained with the aqueous extract of *Drypetes gossweileri*, which was shown to be rich in alkaloids, anthraquinones and saponines, with this extract being efficient against *Acremonium apii* from celery (Ndonkeu et al., 2013). Galani et al. (2013) reported that the antifungal activity of seven Cameroonian plant extracts against *Phythophthora infestans* was due to their richness in phenols, sterols, flavonoids, condensed tannins, coumarins and alkaloids. Phenolic compounds have very high antimicrobial activity (Lapornik et al., 2005) and high activity of coumarins, such as phytoalexins, produced by plants in response to fungal attack has been reported by some authors (Cowan, 1999; Lapornik et al., 2005). However, these findings were not supported by Rashed et al. (2002), who reported high incidence and severity of brown spot only at 50 DAT on the rice hybrids line 321H. This could have been due to the agro-ecological specificities and variety. Aqueous extracts of *E. hirta* at 1kg/15L produced the significantly highest number of panicles (4.76), while the lowest number of panicles was recorded with Artemisia at 2 kg/15 L (2.35) at 110 DAT (Table 7). The application of plant extracts provides minerals and useful metabolites that could improve crop growth and yield. This was reported on beans sprayed with aqueous extracts of *T. vogelii* and *T. diversifolia*, as extracts of these plants contained metabolites, such as rutin, phenylalanine and tryptophan; the effect of these plant extracts was similar to a commercial foliar fertilizer with a significant increase in the yield (Mkindi et al., 2020). Kamalakannan et al. (2001) reported that spraying *Prosopis juliflora* leaf extract was significantly effective in reducing blast in pot culture and field experiments, as well as increasing the yield.

Table 7 Effect of plant extracts application on rice yield variables

Plant extract	Number of panicles		Yield
	100 DAT	110 DAT	(t/ha)
Mancozeb	1.20±0.13^{bab}*	2.84±0.23^{c}	5.6±0.7^{a}
A. annua (67 g/L)	1.11±0.14^{abc}	2.85±0.22^{c}	3.6±0.4^{bc}
A. annua (134 g/L)	0.48±0.11^{d}	2.35±0.18^{c}	2.9±0.2^{c}
C. benghalensis (67 g/L)	0.45±0.13^{d}	2.46±0.25^{c}	3.3±0.3^{bc}
C. benghalensis (134 g/L)	0.58±0.16^{cd}	2.78±0.22^{c}	3.4±0.3^{bc}
E. hirta (67 g/L)	1.51±0.17^{a}	3.81±0.25^{a}	4.5±0.61^{b}
E. hirta (134 g/L)	1.38±0.13^{ab}	4.76±0.27^{b}	5.8±0.9^{a}
Control (water)	0.43±0.18^{a}	2.53±0.23^{c}	3.5±0.4^{bc}

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

However, environmental factors, dosage amount and time of application have also been found to influence the effectiveness of extract applications, regarding both disease management and yield (Kunicki et al., 2010).

Interaction effect between variety and plant extracts on growth, brown spot incidence and yield variables

From the data analysis, the interaction between variety and extracts had a significant effect on the number of leaves for all the weeks measured. The NERICA L56 × *E. hirta* at 67 g/L interaction had the highest number of leaves (39.2), while the LGRR x Mancozeb interaction had the lowest number of leaves (19), as shown in Table 9. NERICA L56 × *E. hirta* at 67 g/L recorded the highest tiller number (13), while LGRR x *C. benghalensis* had the lowest tiller number (5.2). The plant height was greater for the Toukbem x *E. hirta* at 67 g/L interaction (25.8 cm) and lower for the NERICA 8 × *A. annua* at 67 g/L interaction (14.2 cm). The leaf area was the highest for the Toukbem x *C. benghalensis* 67 g/L interaction (32.8 cm^2) compared to the other interactions (Table 9). The Nerica 8 × negative control (water) interaction had the highest occurrence of brown spot, while NERICA L56 × Mancozeb had a lower disease incidence (16.4%), as shown in Table 10. NERICA L56 × *E. hirta* at 134 g/L recorded lower severity (4.3%) while the LGRR × water interaction had the higher disease severity (70%) at 110 DAT (Table 10). The NERICA L56 × Euphorbia at 134 g/L interaction recorded the highest number of panicles (6.06) and yield (5.9 t/ha), which were not significantly different from the NERICA L56 × Mancozeb interaction (6.1 t/ha), as shown in Table 8. These results showed that there was a synergistic effect between plant extract and variety. When the variety NERICA L56 was sprayed with Euphorbia at 134 g/L, the growth and yield variables improved and brown leaf spot incidence was reduced. The synergistic effect of plant extract observed in the current study was also reported in other studies (Rivera et al., 2014; Liu et al., 2016). This may have been due to the *E. hirta* extracts containing flavonoids, phenols and alkaloids known to play very important defensive roles against pathogens. Daniel et al. (2015) showed that the extracts contained different compounds with antifungal properties. The antifungal activity of *Artemisia annua* extracts against *Fusarium oxysporum*, *Fusarium solani* and *Cylindrocarpon destrutans,* agents of root rot disease in crops, was due to the presence of a coumarin derivative present in the plant (Li et al., 2019; Ma et al., 2019).

The results obtained demonstrated that NERICA L56 had a higher yield than NERICA 8 and the local varieties under the experimental conditions. In addition, NERICAL L56 was more tolerant to brown spot disease. The effect of the aqueous extract of *E. hirta* at 134 g/L against brown spot was comparable to Mancozeb at the recommended dose, while the *C. benghalensis* extract was the least effective at 67 g/L. The interaction effect between variety and plant extract improved both the growth and the yield variables of rice and at the same time significantly reduced the brown spot severity. This study highlighted the bioactive properties of aqueous extract of *Euphorbia hirta*, a medicinal Cameroonian weeds, commonly used traditionally to cure diverse livestock and human diseases.

Table 8 Effects of rice varieties and plant extracts interaction on number of panicles

Variety × Plant extract	Number of panicles		Yield (t/ha)
	100 DAT	110 DAT	
Nerica 8 × Mancozeb	0.40±0.27^{efgh}*	1.93±0.39^{efghi}	1.8±0.14^{def}
Nerica 8 × *A. annua* (67 g/L)	0.46±0.27^{efgh}	1.06±0.36^{ghi}	1.3±0.12^{def}
Nerica 8 × *A. annua* (134 g/L)	0.8±0.14^{h}	1.26±0.41^{fghi}	1.5±0.13^{def}
Nerica 8 × *C. benghalensis* (67 g/L)	0.6±0.12^{h}	0.93±0.36^{hi}	1.2±0.1^{def}
Nerica 8 × *C. benghalensis* (134 g/L)	0.46±0.23^{efgh}	2.33±0.4^{defgh}	3.2±0.24^{bc}
Nerica 8 × *E. hirta* (67 g/L)	0.86±0.25^{cdefgh}	3.00±0.42^{cdefg}	3.4±0.51^{bc}
Nerica 8 × *E. hirta* (134 g/L)	0.80±0.27^{defgh}	2.46±0.43^{defgh}	3.3±0.33^{bc}
Nerica 8 × control (water)	0.5±0.11^{h}	0.70±0.26^{i}	1.1±0.04^{def}
LGRR × Mancozeb	1.06±0.32^{cdefgh}	3.26±0.46^{bcdef}	3.1±0.21^{bcd}
LGRR × *A. annua* (67 g/L)	1.20±0.31^{bcdefg}	3.60±0.45^{bcde}	3.7±0.44^{bc}
LGRR × *A. annua* (134 g/L)	0.80±0.22^{defgh}	3.66±0.44^{bcde}	3.6±0.37^{bc}
LGRR × *C. benghalensis* (67 g/L)	0.73±0.27^{efgh}	2.26±0.47^{efgh}	3.1±0.22^{bcd}
LGRR × *C. benghalensis* (134 g/L)	0.93±0.23^{cdefgh}	3.66±0.36^{bcde}	3.7±0.31^{bc}
LGRR × *E. hirta* (67 g/L)	1.93±0.29^{abcd}	4.86±0.46^{abc}	4.3±0.52^{b}
LGRR × *E. hirta* (134 g/L)	1.53±0.27^{abcde}	3.40±0.45^{bcde}	3.5±0.29^{bcd}
LGRR × control (water)	0.33±0.13^{fgh}	3.73±0.32^{bcde}	3.7±0.66^{bc}

Table 8 Effects of rice varieties and plant extracts interaction on number of panicles (Continued)

Variety × Plant extract	Number of panicles		Yield (t/ha)
	100 DAT	110 DAT	
Nerica L56 × Mancozeb	1.00±0.27^{cdefgh}	3.06±0.46^{cdefg}	6.1±0.49^{a}
Nerica L56 × *A. annua* (67 g/L)	2.46±0.22^{a}	4.88±0.33^{abc}	4.4±0.75^{b}
Nerica L56 × *A. annua* (134 g/L)	0.53±0.13^{efgh}	2.13±0.46^{efghi}	2.9±0.18^{de}
Nerica L56 × *C. benghalensis* (67 g/L)	0.73±0.24^{efgh}	3.73±0.47^{bcde}	3.8±0.33^{bc}
Nerica L56 × *C. benghalensis* (134 g/L)	0.60±0.17^{efgh}	2.92±0.46^{cdefgh}	3.3±0.27^{bcd}
Nerica L56 × *E. hirta* (67 g/L)	2.00±0.26^{abc}	6.00±0.44^{a}	5.5±0.92^{a}
Nerica L56 × *E. hirta* (134 g/L)	2.26±0.31^{ab}	6.06±0.37^{a}	5.9±0.83^{a}
Nerica L56 × control (water)	1.26±0.29^{bcdef}	4.33±0.46^{abcd}	4.1±0.72^{b}
Toukbem × Mancozeb	0.73±0.17^{efgh}	3.3±0.43^{cdefg}	3.2±0.22^{bcd}
Toukbem × *A. annua* (67 g/L)	0.33±0.15^{fgh}	1.88±0.35^{efghi}	1.7±0.14^{def}
Toukbem × *A. annua* (134 g/L)	0.60±0.3^{efgh}	2.33±0.41^{defgh}	3.2±0.21^{bcd}
Toukbem × *C. benghalensis* (67 g/L)	0.26±0.14^{fgh}	2.93±0.34^{cdefgh}	3.3±0.22^{bcd}
Toukbem × *C. benghalensis* (134 g/L)	0.33±0.17^{fgh}	2.20±0.38^{efghi}	2.9±0.15^{de}
Toukbem × *E. hirta* (67 g/L)	1.26±0.12^{bcdef}	5.20±0.36^{ab}	4.8±0.65^{b}
Toukbem × *E. hirta* (134 g/L)	0.93±0.13^{cdefgh}	3.5±0.48^{bcde}	3.4±0.11^{bcd}
Toukbem × control (water)	0.13±0.1^{fgh}	1.86±0.47^{efghi}	1.6±0.13^{def}

DAT = days after transplanting; LGRR = Long grain red rice

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Table 9 Effect of rice varieties and plant extracts interaction on plant height, number of tillers and leaves and leaf area of rice at 9 wk after transplanting

Variety × Plant extract	Plant height (cm)	Number of tillers	Leaf area (cm^2)	Number of leaves
NERICA L56 × *Euphobia* 1kg per 15L of water	17.04±1.19^{jklmn}	13.1±3.98^{a}	33.34±7.4^{defgh}	39.2±10.62^{a}
NERICA 8 × *Artemisia* 2kg per 15L of water	21.94±0.9^{bcdefgh}	10.8±2.3^{ab}	29.08±8.65^{efgh}	34.6±8.03^{ab}
NERICA 8 × *Euphobia* 1kg per 15L of water	16.36±2.43^{klmn}	10.6±3.29^{ab}	27.23±7.6^{gh}	35.0±11.77^{ab}
Toukbem × Mancozeb	24.18±1.3^{abcd}	10.4±2.23^{abc}	48.83±15.77^{abcde}	35.4±10.15^{ab}
NERICA 8 × Commelina 2kg per 15L of water	16.56±4.49^{jklmn}	10.2±4.69^{abcd}	23.59±9.63^{gh}	33.6±9.5^{abc}
NERICA 8 × Mancozeb	17.80±1.89^{ijklmn}	9.86±4.88^{abcde}	30.27±10.78^{defgh}	33.9±10.59^{abc}
NERICA L56 × *Commelina* 2kg per 15L of water	20.04±2.10^{efghijk}	9.8±3.02^{abcdef}	33.30±8.36^{defgh}	25.2±6.82^{cdefgh}
NERICA L56 × *Artemisia* 2kg per 15L of water	19.56±2.55^{fghijk}	9.8±0.41^{abcdef}	28.45±8.92^{fgh}	32.2±2.88^{abcde}
NERICA 8 × *Artemisia* 1kg per 15L of water	14.26±1.20^{n}	9.6±2.23^{bcdef}	22.55±6.52^{h}	30.2±9.8^{abcdefg}
NERICA 8 × *Euphobia* 2kg per 15L of water	18.89±2.36^{ghijklm}	9.4±4.47^{bcdefg}	25.99±7.38^{gh}	29.8±10.2^{bcdefg}
Toukbem × *Euphobia* 2kg per 15L of water	20.96±4.13^{defghi}	9.2±1.6^{bcdefg}	49.8±16.83^{abcd}	30.6±2.4^{abcdef}
Toukbem × *Euphobia* 1kg per 15L of water	25.81±0.74^{a}	9.4±1.8^{bcdefg}	64.04±19.13^{ab}	30.2±3.42^{abcdefg}
NERICA L56 × *Euphobia* 2kg per 15L of water	15.64±2.11^{lmn}	9.26±1.71^{bcdefg}	30.96±9.83^{defgh}	27.73±3.8^{bcdefgh}
NERICA L56 × Mancozeb	18.68±2.37^{hijklm}	9.26±1.7^{bcdefg}	33.29±6.49^{defgh}	33.1±3.52^{abcd}
NERICA L56 × *Artemisia* 1kg per 15L of water	20.24±1.85^{efghij}	9.2±2.1^{bcdefg}	34.44±10.83^{cdefgh}	30.4±7.37^{abcdef}
NERICA L56 × Water	18.70±1.72^{hijklm}	8.86±2.5^{bcdefgh}	37.20±16.12^{cdefgh}	29.9±7.1^{abcdefg}
NERICA L56 × *Commelina* 1kg per 15L of water	20.88±1.95^{defghi}	8.8±1.4^{bcdefgh}	35.36±10.89^{cdefgh}	27.7±2.92^{bcdefgh}
Toukbem × *Commelina* 1kg per 15L of water	22.64±2.80^{abcdef}	8.6±2.02^{bcdefgh}	58.79±18.90^{ab}	30.0±6.7^{abcdefg}
NERICA 8 × *Commelina* 1kg per 15L of water	18.35±4.11^{hijklm}	8.4±3.04^{bcdefghij}	27.53±7.27^{gh}	27.4±7.42^{bcdefgh}
Toukbem × Water	20.03±1.9^{efghijk}	8.4±1.5^{bcdefghij}	61.6±17.64^{ab}	29.6±1.8^{bcdefg}
Toukbem × *Commelina* 2kg per 15L of water	22.74±2.1^{abcdef}	8.1±1.9^{bcdefghij}	58.03±21.61^{ab}	28.2±5.4^{bcdefgh}
Toukbem x *Artemisia* 1kg per 15L of water	23.70±1.5^{abcde}	8.0±0.9^{bcdefghij}	65.64±21.52^{a}	27.2±2.1^{bcdefgh}
NERICA 8 × Water	15.42±2.29^{mn}	7.4±2.9^{cdefghij}	28.17±7.47^{gh}	30.8±11.7^{abcdef}
Long grain rice × Negative control	22.78±1.53^{abcdef}	7.0±2.44^{defghij}	61.05±16.84^{ab}	22.8±6.29^{fgh}
Long grain rice × *Artemisia* 2kg per 15L of water	19.32±3.31^{fghijkl}	6.8±1.52^{efghij}	53.96±24.11^{abc}	23.8±3.78^{defgh}
Long grain rice × *Commelina* 2kg per 15L of water	22.76±5.29^{abcdef}	6.6±1.4^{efghij}	58.06±18.8^{ab}	22.6±4.07^{fgh}
Toukbem × *Artemisia* 2kg per 15L of water	21.60±2.13^{cdefgh}	6.46±0.83^{fghij}	43.81±19.81^{bcdefg}	23.6±4.66^{efgh}
Long grain rice × *Artemisia* 1kg per 15L of water	21.62±4.27^{cdefgh}	6.20±0.77^{ghij}	53.88±15.93^{abc}	23.2±1.37^{efgh}
Long grain rice × *Euphobia* 2kg per 15L of water	20.88±2.43^{defghi}	5.60±1.54^{hij}	34.13±12.41^{cdefgh}	24.1±5.59^{defgh}
Long grain rice × *Euphobia* 1kg per 15L of water	25.24±3.54^{abc}	5.60±1.24h^{ij}	49.40±20.21^{abcde}	21.1±3.98^{gh}
Long grain rice × Mancozeb	25.52±3.68^{ab}	5.41±1.54^{ij}	62.65±20.48^{ab}	19.0±3.14^{h}
Long grain rice × *Commelina* 1kg per 15L of water	22.52±1.57^{abcdefg}	5.2±0.77^{j}	60.02±16.18^{ab}	20.2±4.46^{h}

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different; Mancozeb = positive control; and water = negative control

Table 10 Effect of rice varieties and plant extracts interaction on brown leaf spot incidence and severity at 90 d after transplanting, where error bars indicate ± SD

Variety × Plant extract	Disease severity (%)	Disease incidence (%)
Long grain rice × Water	70.01±0.1^{a}	54.14±8.46^{abcd}
NERICA 8 × Water	56.66±2.88^{ab}	64.26±11.97^{a}
Toukbem × Water	50.66±4.61^{abc}	63.47±12.55^{ab}
NERICA L56 × Water	42.66±6.35^{bcd}	58.33±11.71^{abc}
Toukbem × *Artemisia* 1kg per 15L of water	36.33±17.61^{bcde}	39.52±3.59^{abcde}
Long grain rice × *Euphobia* 2kg per 15L of water	31.00±10.39^{cdef}	32.77±2.54^{abcde}
Long grain rice × *Euphobia* 1kg per 15L of water	30.66±1.15^{cdef}	36.66±5.77^{abcde}
Long grain rice × *Commelina* 1kg per 15L of water	30.00±8.66^{cdefg}	46.66±7.63^{abcde}
Toukbem × *Commelina* 1kg per 15L of water	26.66±2.88^{defgh}	29.28±12.01^{cde}
Toukbem × *Euphobia* 1kg per 15L of water	25.00±6.24^{defghi}	27.33±2.51^{cde}
Toukbem × *Commelina* 2kg per 15L of water	20.33±2.88^{efghi}	42.66±17.5^{abcde}
Long grain rice × *Commelina* 2kg per 15L of water	18.33±0.57^{efghi}	37.22±4.98^{abcde}
NERICA 8 × *Commelina* 1kg per 15L of water	17.66±2.51^{efghi}	36.1±11.53^{abcde}
Toukbem × Mancozeb	16.66±5.77^{efghi}	22.40±2.50^{de}
NERICA 8 × *Euphobia* 2kg per 15L of water	16.33±9.01^{efghi}	38.38±7.70^{abcde}
Long grain rice × *Artemisia* 1kg per 15L of water	15.66±0.57^{efghi}	35.00±13.22^{abcde}
NERICA 8 × *Commelina* 2kg per 15L of water	15.33±12.85^{efghi}	30.74±8.91^{abcde}
Long grain rice × *Artemisia* 2kg per 15L of water	15.33±0.57^{efghi}	25.74±3.94^{cde}
Toukbem × *Euphobia* 2kg per 15L of water	13.66±12.05^{fghi}	30.00±5.00^{bcde}
Toukbem × *Artemisia* 2kg per 15L of water	13.33±2.88^{fghi}	39.44±9.18^{abcde}
NERICA 8 × *Artemisia* 1kg per 15L of water	11.66±6.65^{fghi}	45.95±7.96^{abcde}
NERICA 8 × *Euphobia* 1kg per 15L of water	11.66±2.88^{fghi}	37.61±10.97^{abcde}
NERICA 8 × Mancozeb	10.00±8.66^{fghi}	26.66±10.40^{cde}
Long grain rice × Mancozeb	9.66±6.11^{fghi}	20.75±6.16^{de}
NERICA L56 × *Commelina* 2kg per 15L of water	8.33±2.08^{ghi}	36.83±15.89^{abcde}
NERICA L56 × *Commelina* 1kg per 15L of water	7.66±5.85^{hi}	36.90±10.30^{abcde}
NERICA L56 × Mancozeb	6.66±0.57^{hi}	16.42±7.72^{e}
NERICA L56 × *Euphobia* 1kg per 15L of water	6.66±5.50^{hi}	30.74±12.42^{abcde}
NERICA L56 × *Artemisia* 2kg per 15L of water	6.33±4.04^{hi}	46.00±21.51^{abcde}
NERICA 8 × *Artemisia* 2kg per 15L of water	5.33±4.04^{hi}	31.66±7.63^{abcde}
NERICA L56 × *Euphobia* 2kg per 15L of water	4.33±4.93^{i}	48.33±10.4^{abcde}
NERICA L56 × *Artemisia* 1kg per 15L of water	4.00±3.60^{i}	40.23±4.5^{abcde}

Mean ± SD in same column superscripted by different lowercase letters are significantly ($p < 0.05$) different; Mancozeb = positive control; and Water = negative control

Conflict of Interest

The authors declare that there are no conflicts of interest.

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