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QUALITIES OF SOYBEAN GRAINS HARVESTED ON CROPS TREATED WITH NEEM SEEDS EXTRACT IN FIELD CONDITIONS AGAINST ASIAN RUST

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ABSTRACT

In soybean production, qualities of grains harvested are usually affected according to the development of diseases and control methods used in the field. This study aims to evaluate the impact of neem seeds extract (AENS) on soybean grains health after crops treatment in field conditions for the control against Asian rust. Experiment was conducted in Mimetala and Nkometou, located in the agroecological zone V of Cameroun. Three varieties of soybean ("Locale", TGX 1835-10E and TGX1910-14E) were used in a completely randomized blocks design containing five treatments: T₀ (control), T₁, T₂, T₃ (25, 50, 100 g/L of extract respectively) and T₄ (5.33 g/L of Plantineb 80wp). The disease was identified on the harvested grains; its incidence on those grains was evaluated and some biochemical parameters were assessed by extraction and determination of their contents. Many grains were attacked by Asian rust. The highest incidence was obtained in the seeds from untreated plots (35 %), compared to those from T₁, T₂, T₃ (1.14; 1.07 and 0.95 % respectively). The different doses of AENS improved the content of all biochemical compounds tested in harvested grains compared with the T₀ and T₄ treatments. TGX1910-14E variety presented the highest content. In T₃, grain contents in biochemical compounds were 415.17; 118.83; 118.33; 44.43; 36.53 and 10.83 mg/g of dry matter from total proteins, globulin, albumin, Glutelin, prolamin and glucide respectively. Meanwhile, in T₄ treatments, grain contents were 403.49; 112.95; 112.78; 40.58; 33.90 and 9.91 mg/g of dry matter from the same compounds respectively. This study shows that the application of neem seeds extract on soybean crops for the control of Asian rust in field conditions at the maximum dose (e.g. 100 g/L) can improve the qualities of harvested grains. The neem extract as a natural substance should be used in integrated pest management of soybean and other crops.

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INTRODUCTION

Soybean (*Glycine max*), an annual plant of the Fabaceae family, is present in more than 80 countries worldwide. Native from East Asia (Qiu and Chang, 2010), it is grown mainly for its highly rich seeds in protein (40%), fat (20.2%) and trace elements (Labat, 2013). The world production of soya increased considerably during the

20th century. According to the FAO, in 2010 the soybean production was 264.18 million tons and in 2017 it increased to approximately 336 million tons on an acreage of more than 1 million km². The main producers are United States, Brazil and Argentina with about 82%. However this very high productivity results from the increase of cultivated surfaces inducing large

deforestation. In South America, cultivated surfaces passed from 18 to more than 40 million hectares. Today, in Cameroon and many other African countries, soybeans are widely used in various forms of food to curb malnutrition and for animal nutrition. However, multiple infections substantially reduced the productivity of soybean. Several fungal diseases affect many varieties of soybean in the field such as anthracnose, sklerotinosi, Phytophthora root rot. Besides, the Asian rust is nowadays the most widespread disease throughout the world (Wrather *et al.*, 2010). Asian Rust must be introduced with host range like soybean and other Fabaceae herbaceous plants. Symptoms can appear on all parts of the plant, but they are more present on leaves in lower plant canopy. Tan or reddish-brown spots develop first on the underside of leaves. Small blisters develop in the lesions which break open and release masses of tan spores (Goellner *et al.*, 2010). Asian Rust caused by *Phakopsora pachyrhizi* and *P. meibomia* can attack young or old plants and may occur yield losses around 80% in absence of any control method (Giller, 2007). Two main methods of control are used to remedy these pathologies, namely genetic and chemical control. These individual methods have shortcomings and are harmful to the environment (Ambang *et al.*, 2007). Improved varieties used in genetic control do not unfortunately totally resist against these diseases. In addition, chemical control is the most used and most effective; based primarily on the use of synthetic pesticides it is costly and harmful because of the concentration of residues in food chains (Essou Ngando *et al.*, 2006). It could inhibit the synthesis of biochemical compounds which play a very important role in the physiology and metabolism of plant (Siddiqui and Ahmed, 2006). An alternative method, the use of environmentally friendly biopesticides is conducive to healthy and sustainable agriculture. In the search of natural pesticides, neem (*Azadirachta indica*) is one of the most widely used plants. This plant of the Meliaceae family is originally from eastern India and the pesticide potential of the extracts has already been demonstrated on several plants. Thus, it has fungicidal (Sharma *et al.*, 2003; Mboussi *et al.*, 2016), insecticide (Awad and Shimaila, 2003; Da-Silva and Martinez, 2004), and nematocidal (Javed *et al.*, 2007; Kosma *et al.*, 2011) properties. Its effectiveness in the fight against Asian rust of soybean would enhance the protection of plants in the field and

the environment. The main objective of this study is to evaluate the effect of aqueous extract of neem seeds on the qualities of harvested soybean grains.

MATERIALS AND METHODS

Characterization of study sites

The study was carried out at Mimetala and Nkometou two localities in the agroecological zone V in Cameroon, characterized by a bimodal rainfall. The average annual rainfall in this agroecological zone varies between 1500 and 2000 mm; the average temperature is between 23 and 27 °C; the average relative humidity is above 80% (Moundigo, 2007). Mimetala is located in forest part and Nkometou is in transition zone of savannah to forest.

Plant materials

The plant material consisted of three varieties of soybean and neem seeds. The soybean varieties used were constituted by one "Locale" and two improved (TGX1835-10E and TGX1910-14E). The "Locale" variety was bought in a market of Yaounde town coming from local farmers. The TGX1835-10E and TGX1910-14E varieties were obtained respectively from the Institute of Agricultural and Development Research (IADR) of Fombot and Garoua, cultivated in normal seed reproduction plantation. The ripe neem fruits were collected on the ground under neem trees in the Northern region of Cameroon. Chemical material used was the Plantineb 80WP which is a contact fungicide and contains 80% of Maneb (Ditiocarbamat) and was applied at the recommended dose: 5.33 g/l.

Preparation of extract and application on plants

Aqueous extract was obtained using the method of Kumar (2003) based on maceration. Mature fruits harvested in the northern part of the country were broken to collect the seeds. These were then dried at ambient temperature (25±2 °C) for 2 to 3 days and crushed. To obtain the three concentrations used (C₁, C₂ and C₃): 250, 500 and 1000 g of neem seeds powder were introduced respectively in 10 L of tap water, kept for about 12 hours in ambient conditions of laboratory and filtered with the muslin tissue. These solutions were directly applied in the field by adding ten (10) g of soap powder (Detergent) used as wetting. The solution of Plantineb 80WP was used at the recommended concentration (5.33 g/L). Spraying was made weekly for the extract and the chemical fungicide. The aqueous extracts of neem seeds were applied at the dose of 25 kg/ha and the fungicide at 2.5 Kg/ha. First application

was done at the 4th week after sowing (WAS) and five applications were taken before grain harvest.

Experimental design

The experimental design used in each site was a system of completely randomized blocks containing five (05) treatments. T₀ (control plants treated with simple water); T₁, T₂, T₃ treatments containing respectively a solution of 25, 50 and 100 g/l of extract and T₅ (5.33 g/l of Plantineb 80 wp). All treatments were repeated three times.

Identification of the disease on the harvested grains

On field, Asian rust appeared both in leaves and soybean pods. During the harvest of ripe pods, all pods with typical symptoms of Asian rust, were collected. On these diseased pods, cockles were removed; grains were bagged and returned to the laboratory for pathogen identification.

Infected grains were incubated on PDA medium and the pure strains were obtained. The identification of *Phakopsora pachyrhizi*, causal agent of Asian rust achieved according to methodology used by Renard and Foucart (2008) and Shahbaz *et al.* (2009).

Evaluation of different farm treatment effects on the development of disease on harvested grains of soybean

During harvesting, pods were collected, counted and sacked per plant of soybean, grouped per plot, and then the sachets were put in bags initially labeled. The average number of pods were counted per plant. Infected pods were selected and its cocks were removed. Diseased grains were counted per pod and plant, according to their different treatments (T₀, T₁, T₂, T₃ and T₄), varieties and sites.

The average number of harvested grains per pod and treatment was also recorded in order to calculate the incidence. It was determined using following formula of Chumakov and Zaharova (1990):

$$I (\%) = \frac{Nd}{Nt} \times 100$$

Where: Nd = Number of diseased grains; Nt = Total number of grains; I = Incidence in percentage (%)

Extraction and assay of total and reserve proteins

Total proteins were extracted according to the modified Zerrad *et al.* (2008) method. Each specimen of soybean powder (1 g) was crush in a mortar with acetone, then blended with 3 ml of tris 0.1 M (pH 7.1)

containing β mercaptoethanol at 1 % (v/v) and glycerol at 5 % (v/v).

Four reserve proteins were extracted and assayed. Those proteins were extracted according to the modified method of Voigt *et al.* (1993). To avoid an irreversible denaturation of storage proteins by oxidation products of polyphenols during extraction, the acetone dry powder (Ac DP) was extracted each time at 4 °C successively with 10 mM tris-HCl (pH 7.5; 2 mM EDTA), 0.5 M (v/v) ethanol and 0.1 N NaOH. These steps permitted to obtain the albumin, globulin, prolamin and glutelin fractions respectively. Centrifugation was realized at 6000 rpm for 30 mn. All these solvents contained 5 mM sodium ascorbate.

In each case, protein concentrations were determined according to Bradford (1976) using bovine serum albumin as the standard. The absorbance was measured by a spectrophotometer at 595 nm.

Glucide extraction and assay

Extraction of total sugars (glucide) was carried out by grinding in the ethanol-water mixture: 80-20 (v/v), 0.1 g of each sample of soybean grains. The ground materials thus obtained were centrifuged at 4500 g at 4 °C. for 15 minutes (Zerrad *et al.*, 2008). The total sugars concentrations were obtained using enthrone method of Yemm and Willis (1954) in reference to a standard curve made with glucose. The amounts are expressed in mg/g of dry matter.

Statistical analyses

The data were subjected to tests of analysis of variance. After rejecting null hypothesis of equal means using ANOVA, Duncan-test was used for comparing treatment group means at P= 0.05. Statistical software used was SPSS 20.0 for windows and graphs were obtained using Excel spreadsheet of Microsoft 2016 program.

RESULTS

Symptoms observed on the harvested grains

At harvest, some basal pods and seeds were covered with a thin mycelial layer of whitish color and a cottony appearance compared to normal phenotype of grains (Figure 1A and 1B). Microscopic observation of the pure strains isolated from those diseased grains allowed to highlight the presence of numerous uredospores of various sizes; characteristics of *Phakopsora pachyrhizi*, causal agent of Asian rust (Figure 1).

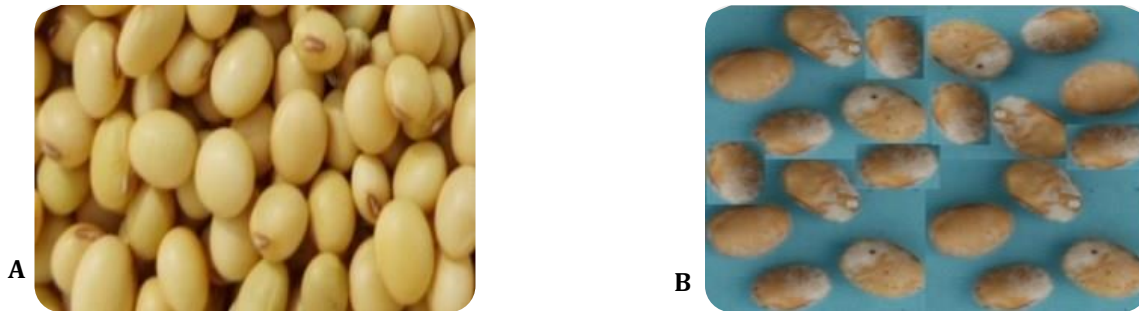


Figure 1. Phenotype of harvested grains: A- normal grains, B- diseased grains.

Effect of farm treatments on the development of Asian rust on harvested soybean grains

Results showed that in both sites after harvesting, “Locale” variety presents a high number of diseased grains. On grains from Mimetala for example, that variety presented a disease incidence of 1.34 % in the control and 0.95 % in treatments T₃ (100 g/l of extract) and T₄ (Chemical). That variety was followed by

TGX1910-14E which presents an incidence of 1.25 % in the control and 0.95 % in T₃ and T₄ treatments. The grains of TGX1835-10E were the least attacked in all treatments and all sites. Globally, incidence of disease proportionally varied on the grains with different doses of extract tested in the farm (Figure 2). Statistical analysis showed that there is no significant difference between T₃ and T₄ treatments in both sites ($p \leq 0.05$).

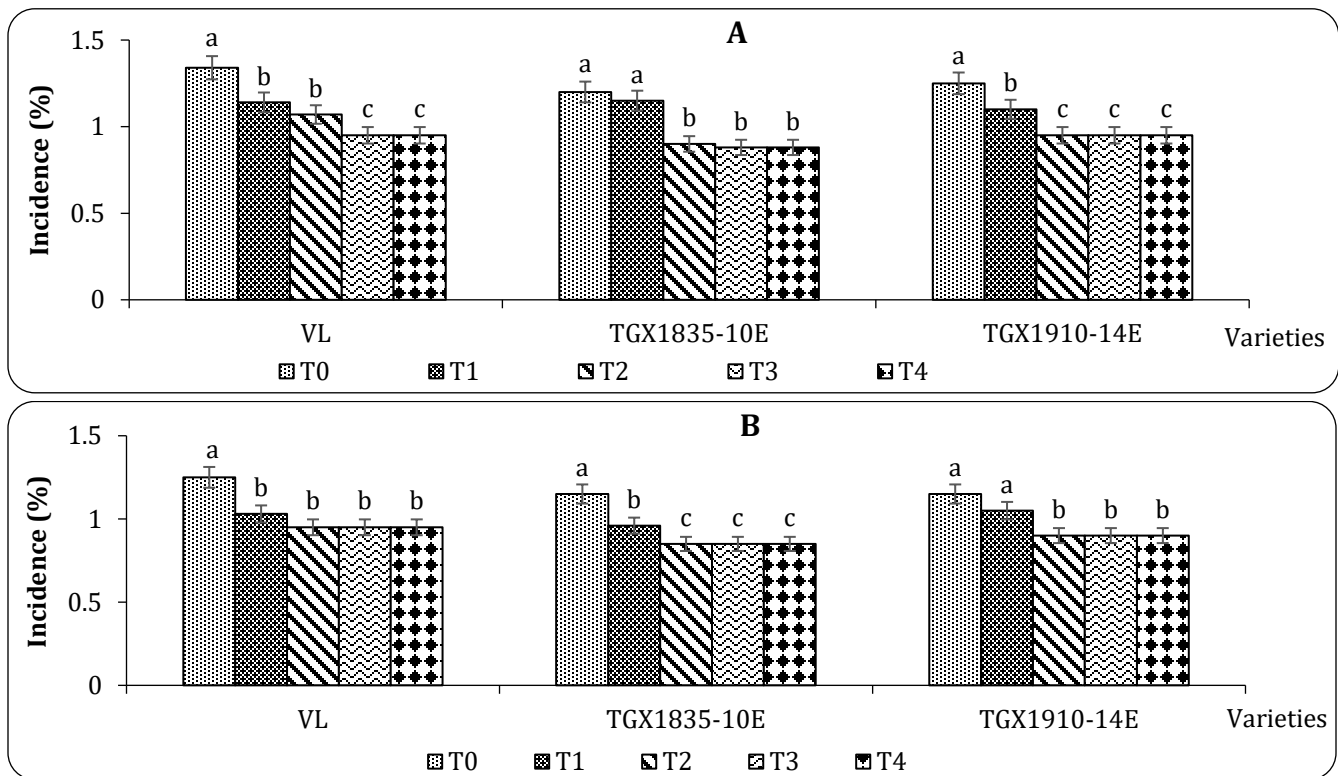


Figure 2. Incidence of the disease in the harvested grains: A- from Mimetala, B- from Nkometou, T₀, T₁, T₂, T₃-treated with water; 25 ; 50 and 100 g/l of neem extract in the farm respectively, and T₄- chemical fungicide).

Effect of extract on the total protein content of soybean seeds

In grains from both sites, TGX1910-14E variety presented the highest total protein content, followed by

TGX1835-10E, the “Locale” proved to be the least rich. In addition, it was observed that for all varieties and in the experimental sites, the total protein content of soybeans varied with the different treatments. However, it was

noticed that this content was higher in the seeds resulting from the T₃ treatment (415.77; 412.55 and 411.69 mg/g of DM respectively from TGX1910-14E, TGX1835-10E and "Locale" varieties) compared to that of seeds resulting from T₄ (403.49; 401.88 and 400.96

mg/g of DM respectively from the same varieties).

Statistical analysis showed that for all varieties tested, there is a significant difference ($p \leq 0.05$) between T₃ treatment and all others, and even between the three soybean varieties used (Figure 3).

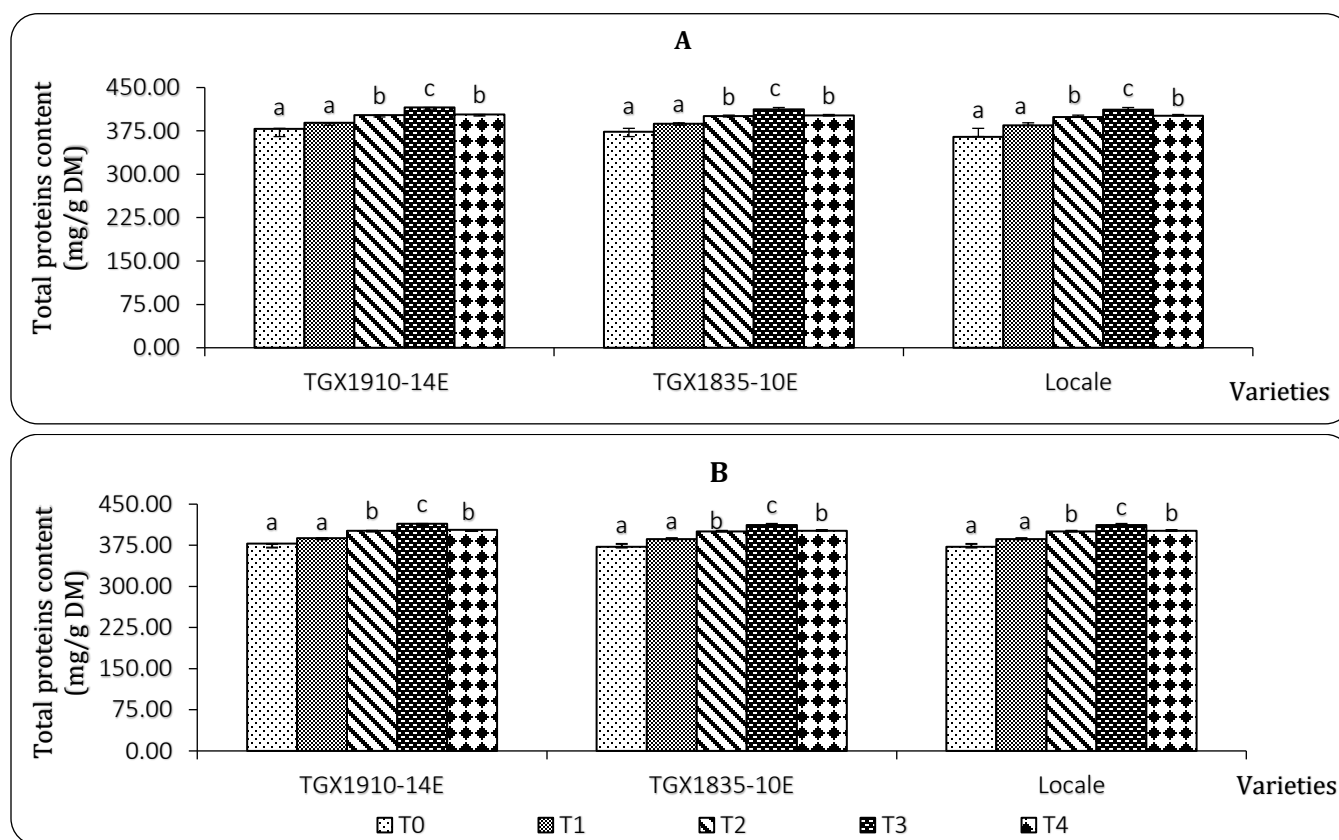


Figure 3. Total proteins of harvested grains: A- Mimetala grains; B- Nkometou grains; T₀, T₁, T₂, T₃- treated with water, 25; 50 and 100 g/l of AENS respectively and T₄- treated with chemical fungicide).

Effect of extract on reserve proteins content

The reserve proteins have been classified as Albumin, Globulin, Prolamin and Glutelin. The results of the present study showed that soybean grains from both sites are very rich in Globulin and Albumin; Prolamin and Glutelin are poorly represented. Among all varieties used, TGX190-14E was the richest in reserve proteins, followed by TGX185-10E; "Locale" variety being the least rich.

Effect of extract on Globulin content

Among the four classes of specific reserve proteins, Globulin was the most represented for all soybean varieties used. Its content increased with the different concentrations tested in the field with the lowest values presented by grains from untreated plots. In both sites, the highest content was obtained with the seeds resulting from the plots treated with 100 g/l of extract

(T₃) followed by those from plots treated with chemical fungicide (T₄). In grains from Mimetala, T₃ grains presented the values of 118.93; 112.95 and 107.95 mg/g of DM respectively from TGX1910-14E, TGX1835-10E and "Locale" varieties, followed by those resulting from the T₄ treatment (114.25; 107.65 and 101.95 mg/g of DM respectively from the same varieties). The statistical analysis showed that there is a significant difference between the Globulin content of seeds from T₃ treatments and all other treatments, and no significant difference ($p \leq 0.05$) between T₂ and T₄. Overall Globulin content varied very little with different sites. Variety TGX1910-14E was among the three varieties used, the richest in Globulin regardless of the concentrations tested and even sites (Figure 4).

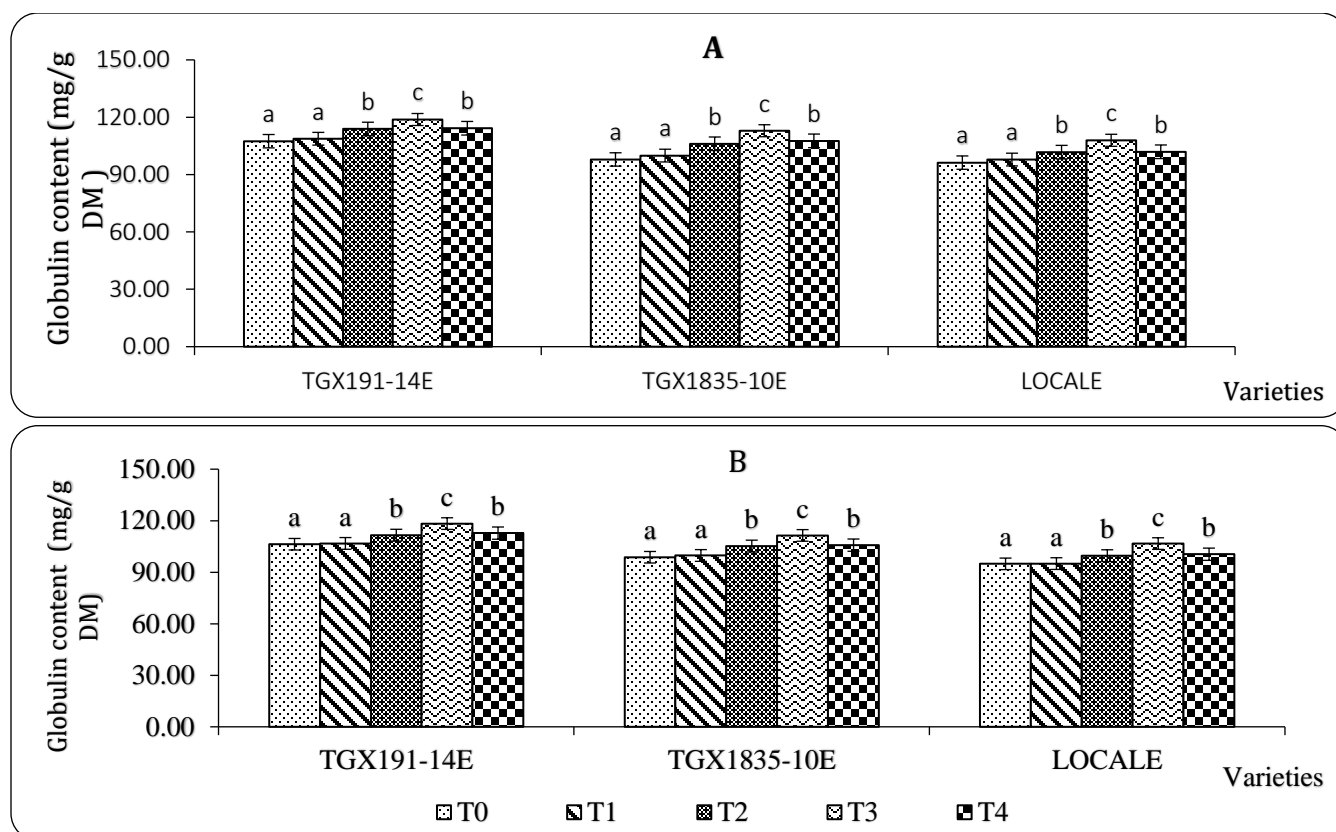


Figure 4. Variation of Globulin content in soybean grains tested : A - from Mimetala ; B- from Nkometou ; T0, T1, T2, T3 – treated with water; 25 ; 50 et 100 g/l of AENS respectively ; T4- chemical fungicide.

Effect of extract on albumin content

Albumin was the second most important reserve protein after Globulin in all varieties and seeds from all field treatments in both sites. As in the case of Globulin, the results showed that, the highest albumin content was obtained with seeds from the T3 treatment (100 g/l of extract) with the values of 117.33; 111.43 and 106.68 mg/g DM respectively from TGX1910-14E, TGX1835-10E and “Locale” varieties. The following value was obtained with seeds from plots treated with chemical fungicide T4 (112.78; 105.78 and 100.48 respectively from the same varieties) treatment. Statistical analysis showed that there is a significant difference ($p \leq 0.05$) between T3 treatment and all other treatment (Figure 5).

Effect of extract on Glutelin and prolamin content

This study presented Prolamin and Glutelin as, unlike Albumin and Globulin, both least important reserve proteins in soybean, regardless of varieties, the different treatments performed in the field an even the sites. Grains from untreated plots presented the lowest contents of both compounds compared to other treatments.

Glutelin content

Glutelin was lowly represented, nevertheless, its content varied according to the different treatments carried out in the field in both sites (Table 1). From Mimetala grains, their higher content was obtained with seeds from plots treated with 100 g/l of extract (44.43; 43.58 and 41.93 from TGX1910-14E, TGX1835-10E and “Locale” varieties respectively), followed by grains from plots treated with chemical fungicide T4 (40.58; 39.85 and 37.48 respectively from the same varieties).

Prolamin content

Prolamin was the least represented reserve protein between the two. Nevertheless, the reserve protein also varied according to the different treatments carried out in the field (Table 2). With grains from Mimetala, the higher content was obtained with seeds from plots treated with 100 g/l of AENS T3 (36.53; 27.93 and 24.78 respectively from TGX1910-14E, TGX1835-10E and “Locale” varieties), followed by those from plots treated with chemical fungicide T4 (33.90; 23.13 and 20.40 respectively from the same varieties) (Table 2).

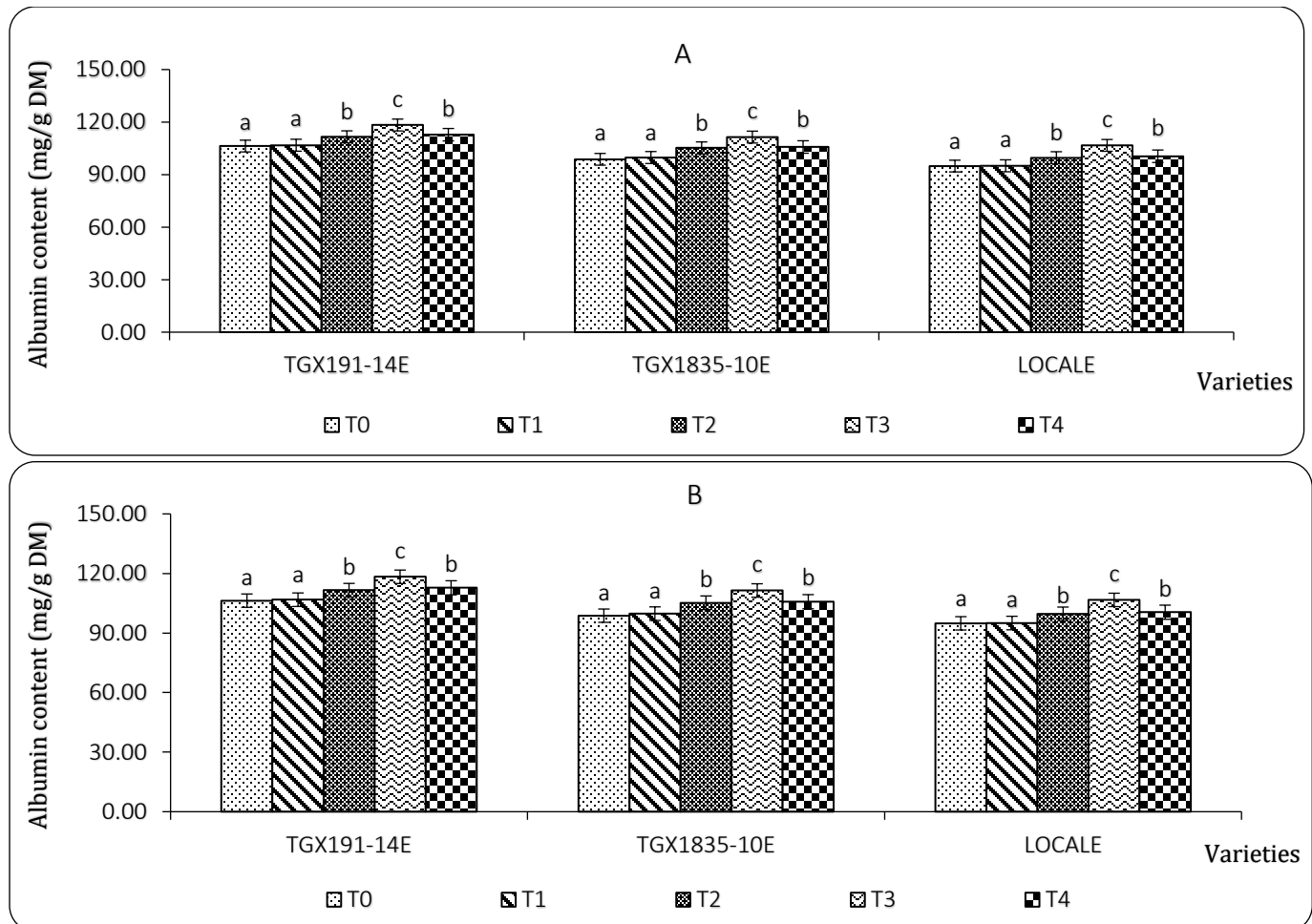


Figure 5. Variation of Albumin content in soybean grains tested : T0, T1, T2, T3 - treated with water; 25 ; 50 et 100 g/l d'AENS respectively; T4- chemical fungicide.

Table 1. Variation of the Glutelin content (mg/g DM) in the seeds according to the different defensive substances and varieties tested: T0, T1, T2, T3- plots treated with water; 25, 50 and 100 g / l of EANS respectively; T4 plots treated with chemical fungicide.

		GLUTELIN (mg/g DM)		
		Varieties		
Study sites	Treatments	TGX1910-14E	TGX1835-10E	Locale
MIMETALA	T0	35.88 a	35.53 a	34.23 a
	T1	36.90 a	35.98 a	34.60 a
	T2	39.73 b	38.68 b	37.38 b
	T3	-	43,58 c	41.93 c
	T4	40.58 b	39.85 b	37.48 b
NKOMETOU	T0	35.70 a	35.4 a	33.68 a
	T1	36.65 a	35.63 a	33.85 a
	T2	39.48 b	38.73 b	35.98 b
	T3	43.10 c	42.33 c	39.80 c
	T4	40.30 b	39.65 b	35.63 b

Table 2. Variation of the Prolamin content (mg/g DM) of the seeds according to the different defensive substances and varieties tested: T0, T1, T2, T3- plots treated with water, 25, 50 and 100 g / l of EAGN respectively; T4- plots treated with chemical Fungicide.

		PROLAMIN (mg/g DM)		
		TGX1910-14E	TGX1835-10E	Locale
MIMETALA	T0	27.00 a	20.90 a	16.88 a
	T1	28.08 a	21.13 a	17.03 a
	T2	31.13 b	23.03 b	19.95 b
	T3	36.53 c	27.93 c	24.78 c
	T4	33.90 b	23.13 b	20.40 b
NKOMETOU	T0	25.75 a	20.73 a	16.48 a
	T1	27.55 a	20.93 a	16.95 a
	T2	31.93 b	24.53 b	19.60 b
	T3	35.23 c	26.73 c	23.48 c
	T4	32.35 b	23.98 b	20.15 b

Effect of disease and extract on total sugar (Glucide) content of seeds

Quantitative analysis of total sugars in the different varieties of soybeans used showed that, overall, soybean has relatively low sugar content. In addition, it was observed that, in the absence of any treatment, the accumulation of total sugars is greater in the variety TGX1910-14E, followed by the variety TGX1835-10E. The local variety was the least rich in total sugars. Nevertheless, the total sugar content varied with the different concentrations of extract tested in the field, with the lowest content obtained with the grains from untreated plots. In grains from Mimetala, this content was higher in the seeds from plots treated with 100 g/l of extract T3 (10.83; 10.73 and 10.52 respectively from TGX1910-14E, TGX1835-10E and 'Locale' varieties) (Figure 6), followed by that of the seeds resulting from the plots treated with chemical fungicide T4 (9.91; 9.61 and 9.61 respectively from the same varieties). There is a significant difference between these two treatments on the one hand and between T3 and all other treatments (Figure 6).

DISCUSSION

The evaluation of the effects of different farm treatments of aqueous extract of neem seeds (AENS) on soybeans revealed that grains from untreated plots were more infected by Asian rust caused by *Phakopsora pachyrhizi* than those from other treatments. This shows that neem extract helps to reduce the evolution of Asian rust on the

harvested grains. This result is similar to those obtained by Mweshi *et al.* (2010) who, working on the effect of plant extract such as neem against soybean diseases, showed that neem extracts help to reduce disease that affect grains. We can notice that AENS protect soybean not only on the field during the growth, but harvested grains can also have better health. This statement was demonstrated by Ambang *et al.* (2011) in similar study when using aqueous extract of *Thevetia peruviana* against groundnut leaf spot.

During their development, soybeans accumulate huge amounts of protein and many other substances such as carbohydrates. The results of this study showed that disease, variety and different treatments of AENS influenced the total and reserve protein and even total sugar content of soybean grains. Indeed, for all biochemical compounds tested, grains from untreated plots presented the lowest contents. The variety TGX1910-14E, regardless of treatments and even sites, was the richest in total protein, followed by the variety TGX1835-10E, local variety proved to be the least rich. This could be explained firstly by the fact that TGX1910-14E and TGX1835-10E varieties were the least affected by the disease, which would justify the negative influence of disease on the biochemical composition of grains. On the other hand, these two varieties are initially improved, and naturally different each other and also different from local variety. As shown by Fromme *et al.* (2017) with sorghum grains, biochemical composition of grains would vary from one variety to another, regardless of the different treatments applied in the field.

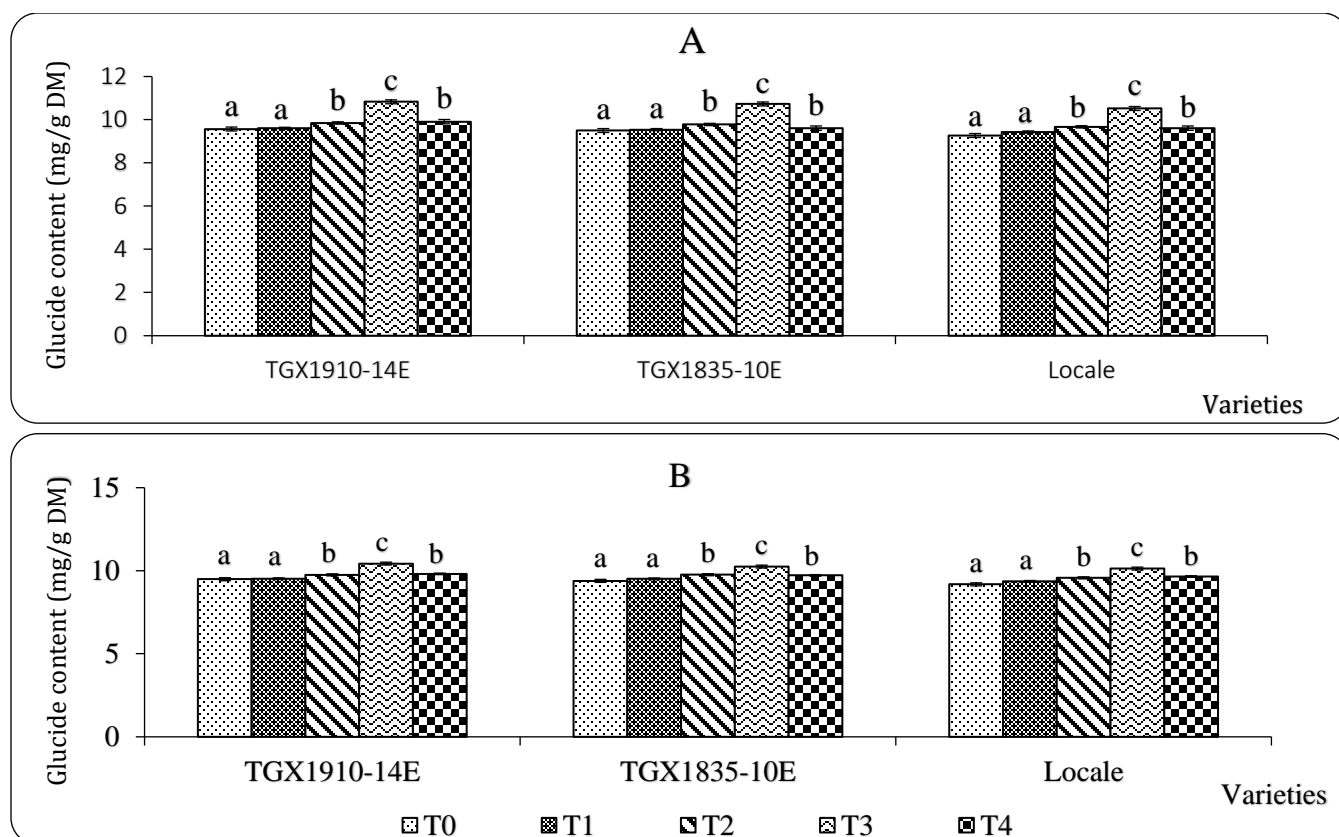


Figure 6. Variation of total sugar content in soybean grains tested: A- from Mimetala ; B- from Nkometou ; T0, T1, T2, T3- Plots treated with water; 25 ; 50 et 100 g/l of AENS respectively; T4- plots treated with chemical fungicide.

In addition, it was observed that for all varieties and at all sites, total and reserve protein contents of soybean grains, varied with different treatments. For these nutritive values, analysis showed a significant difference between grains from untreated plots and those from the other treatments. However, it was noticed that protein content was higher in grains from T₃ treatment (treated at the highest dose of AENS: 100 g/l) compared to that of T₄ treatment (treated with chemical fungicide). Chemical fungicides, used at relatively high doses, would inhibit the process of synthesis and accumulation of biochemical compounds by seeds. Siddiqui and Ahmed (2006) after similar work, had shown that on plots treated with low concentrations of chemical pesticide, protein content of soybean grains was very high. However, with increasing concentrations, these levels gradually decreased.

The same observation was made with carbohydrate content. In fact, it was noticed that carbohydrate was also higher in grains from T₃ treatment compared to that of T₄ treatment. Chemical fungicides, used at relatively high doses, would also decrease carbohydrates content

of soybean grains. Parween *et al.* (2012) have shown that chlorpyrifos insecticide application at high concentrations reduced soluble carbohydrate content of *Vigna radiata* L.

CONCLUSION

In the present study realized in Cameroon, the use of aqueous extract of neem seeds in field conditions, reduced the development of Asian rust not only on the crops during the growing period, but also on the soybean grains after harvest. According to the varieties and the doses of neem seed extracts, the content of biochemical compounds in soybean grains (total proteins, reserve proteins, globulin, albumin, Glutelin, prolamin and glucide) were improved in comparison with the control treatment. In contrary, results showed that chemical fungicides, used at relatively high doses, can inhibit the synthesis and accumulation of biochemical compounds in soybean grains. The results obtained in each study site, show that neem seed extracts are able to improve the qualities of soybean grains. Looking to the simple process of their obtaining,

aqueous extracts of neem seeds should be considering as a promoting bio substance, which can be used in integrated methods of control against Asian rust in soybean production in Cameroon.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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