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Mihikouwè Mazinagou
Department of Soil Sciences, Advanced Agronomy School of University of Lomé, Togo

Jean Mianikpo Sogbedji
Department of Soil Sciences, Advanced Agronomy School of University of Lomé, Togo

Atchala N'Gbendema
Department of Soil Sciences, Advanced Agronomy School of University of Lomé, Togo

Corresponding Author:
Mihikouwè Mazinagou
Department of Soil Sciences, Advanced Agronomy School of University of Lomé, Togo

Zinc biofortified maize (*Zea mays* L.) grain production strategy on the rhodic Ferralsol of Southern Togo

Mihikouwè Mazinagou, Jean Mianikpo Sogbedji and Atchala N'Gbendema

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Abstract

Micronutrient deficiency is a major strain to increasing food production and a global concern for human nutrition. To help meet this challenge, a study was carried out over two consecutive years (2020 and 2021) at the University of Lome Agronomic Experiments Station with the aim to increase productivity and zinc content in maize grains. The experiment was set up in a split-plot design composed of twelve (12) treatments in three replications. Four maize varieties (V_1 = Ikenne, V_2 = Tzee, V_3 = Sotubaka and V_4 = Sammaz 52), and three zinc doses ($Zn_0 = 0$ kg ha^{-1}; $Zn_{45} = 3.75$ kg ha^{-1} and $Zn_{60} = 5$ kg ha^{-1} of ZinGap at 12% Zn) were used for this experiment. Yields and grain zinc concentration were determined. The results showed that maize grain yield and zinc concentration in maize grain were significantly influenced by varieties and zinc doses. On 2-year average basis, the highest yield (3.81±0.13 t ha^{-1}) were recorded under Sotubaka with the application of Zn_{60}. Globally, maize grain yield got under Zn_{60} were 15.13 and 3.65% higher than those obtained under Zn_0 and Zn_{45} respectively. Based on 2-year average, the highest zinc concentration was recorded in Sotubaka grains (55.62±3.78 mg kg^{-1}). This concentration is higher than those obtained in Ikenne, Tzee and Sammaz 52 grains by 17.02; 7.13 and 2.64% respectively. 2-year average zinc concentration obtained under Zn_{60} (66.09±2.31 mg kg^{-1}) was higher than those recorded under Zn_0 and Zn_{45} by 118.12 and 9.10% respectively. On 2-year average basis, the Zn_{60} application to the Sotubaka (70.58±2.91 mg kg^{-1}) gave the highest zinc concentration. Zinc foliar application improved yield and zinc content in maize grain; but its effectiveness depends on environmental conditions.

Keywords: Maize variety, zinc doses, grain, yield, zinc concentration

Introduction

Agricultural production in Sub-Saharan Africa presents numerous biotic and abiotic strains, including micronutrient deficiencies. Among micronutrients, zinc (Zn) deficiency is considered a major threat to global and regional food security (Rana and Kashif, 2014) [50], because it is the most deficient micronutrient in soils worldwide (Cakmak, 2002; Shivay *et al.*, 2008) [11, 55] and over 30% of soils have low Zn availability (Gibson, 2006; Alloway, 2008) [22, 4]. Zn deficiency is therefore a major strain to food production and a global concern for human nutrition (Farooq *et al.*, 2018) [21]. Importantly, the geographic distribution of Zn deficiency in the human diet overlaps with that of Zn deficiency in soil (Peramaiyan *et al.*, 2022) [47]. Furthermore, Zn is an important micronutrient for healthy plant growth and development (Peramaiyan *et al.*, 2022) [47]. It plays multiple roles in fundamental biochemical processes in plants, including enzyme activation, protein synthesis, starch, auxin and nucleic acid metabolism, and pollen development (Marschner, 1995; Cakmak, 2000; Chang *et al.*, 2005) [44; 10; 15]. Zn deficiency in plants leads to disruption of enzyme functions, protein synthesis and impairs plant growth (Akram *et al.*, 2017) [2]. In addition, Zn deficiency in humans was the fifth leading cause of disease and death in developing countries (White and Broadley, 2009) [63] particularly affecting young children and women of child-bearing age (Brown *et al.*, 2004, Groote *et al.*, 2016) [9, 24]. It could affect human health by impairing growth, immune system function, mental health and sexual maturation (Andreini *et al.*, 2006; Gibson, 2012; Krężel and Maret, 2016) [6, 23, 36]. Inadequate dietary intake of Zn was identified as the main reason for this global problem, particularly among the majority of people in developing countries, whose diets were mainly based on wheat.

(Cakmak *et al.*, 2010) [13]. The main reason for Zn deficiency in humans was over-reliance on low-Zn foods, particularly cereals.

Traditional interventions such as supplementation, food fortification and dietary diversification have not achieved the desired success for many reasons (Veni *et al.*, 2019) [60]. Thus, scientists turned more to agronomic biofortification of zinc in food crops to improve human bioavailability of zinc and maintain crop yields, as this type of biofortification was more adaptable and accessible to rural populations (Cakmak, 2008) [12]. Agronomic biofortification of food crops contributed to solving global food security and human nutrition problems on a sustainable basis (Akram *et al.*, 2020) [3]. It aims to increase the concentration of this micronutrient in the edible parts of staple crops without adversely affecting yield (Kachinski *et al.*, 2020) [32]. However, foliar application of Zn is much more effective than soil application of Zn in enriching crop grains with Zn (Wang *et al.*, 2012; Zhang *et al.*, 2012) [62, 67], as the availability of soil Zn to plants depends on soil and climatic factors (Veni *et al.*, 2019) [60].

In Sub-Saharan Africa, maize (*Zea mays* L.) is a staple food consumed by the majority of people (Macauley & Ramadjita, 2015) [41]. It is one of the main food crops in Togo; but its cultivation was characterized by low yields whose national average has never exceeded 1.50 t.ha^{-1} since 2010 (DSID, 2022) [19]. It should also be noted that the maize grains produced intrinsically contain very little Zn to satisfy daily human needs, particularly when maize is grown on Zn-deficient soils (Cakmak, 2008) [12]. This low Zn content in maize grains can lead to Zn deficiency in humans, who are highly dependent on it. It is therefore of great interest to increase Zn concentrations and bioavailability in grain, in order to effectively combat Zn deficiency in humans and reap the benefits for human health. Thus, agronomic practices aimed at zinc biofortification of maize grains are important to mitigate zinc deficiency in humans, especially in resource-poor people due to their diets dominated by cereal-based foods with low zinc concentration and bioavailability (Xia *et al.*, 2018) [65]. That is the background of this study, which aims to: (i) assess the effect of foliar application of zinc on maize grain yield; (ii) determine the most Zn accumulated variety in maize grains and (iii) assess the effect of varieties and zinc doses interactions on zinc concentration in maize grain.

Materials and Methods

Experimental site

The study was carried out at the Lomé Agronomic Experiments Station, located at the University of Lomé - Togo (6°22' N, 1°13'E; altitude of 50 m, slope less than 1%). The soil type was a rhodic Ferralsol locally called “Terres de barre”, developed from the continental deposits (Saragoni *et al.*, 1992) [53]. This type of soil represented 47% of the soils of the maritime region (Worou, 2000) [64] and covered part of the arable land in Ivory Coast, Ghana, Togo, Benin and Nigeria (Raunet, 1973; Louette, 1988) [51, 40]. The climate of the experimental site was the Guinean type, bimodal and allowed for two maize cropping seasons, one from April to July and another from September to December (Sogbedji *et al.*, 2017) [56]. Annual rainfall at the site was between 800 and 1100 mm (Adewi; 2010) [1]. The annual average temperature was between 24 and 27°C (Worou, 2000; Somana *et al.*, 2001) [64, 57].

The experimental plot was under fallow for three years. Before the maize sowing in April 2020, initial soil properties including total C and total N levels, exchangeable base concentrations (Ca^{++}, Mg^{++}, Na^{+} and K^{+}), pH, cation exchange capacity (CEC) and soil texture were determined on the first 20 cm soil layer (0-20 cm depth) on the experimental site. It was done through twenty-four (24) composite soil samples obtained by using the standard methods of the International Institute for Tropical Agriculture (IITA, 2014) [27]. These composite soil samples were analysed at the Laboratory of Soil Water Plant Fertilizer of the Togolese Institute for Agronomic Research (LSEVE-ITRA) and in the Geochemistry and Environment Laboratory (LGE). Total C and total N levels, exchangeable base concentrations (Ca^{++}, Mg^{++}, Na^{+} and K^{+}), pH, cation exchange capacity (CEC) were determined. The soil of the experimental site was slightly acidic (pH=6.12) and had low total C (0.59%) and total N (0.05%) levels. It was sandy and contained 81.05% sand, indicating that this soil was well drained with low contents of P (7.29 mg kg^{-1}); K (22.44 mg kg^{-1}) and Fe (42.84 mg kg^{-1}) contents. Its CEC was very low 2.82 mmol kg^{-1}, as were exchangeable bases Ca $^{++}$, Mg^{++}, Na^{+} and K^{+}, with respective values of 41.42; 5.98; 1.17 and 0.57 mmol kg^{-1} (Table 1).

Table 1: Soil physical and chemical properties at onset of experiment

Parameters	Values
pH (H_2O)	6.12
Organic matter (%)	1.02
C total (%)	0.59
Total N (%)	0.05
NO_3-N (mg kg^{-1})	1.92
P available (mg kg^{-1})	7.29
K available (mg kg^{-1})	22.44
Fe total (mg kg^{-1})	42.84
Zn total (mg kg^{-1})	14.22
Exchangeable bases (mmol kg^{-1})	
Ca^{2+}	41.42
Mg^{2+}	5.98
Na^{+}	1.17
K^{+}	0.57
CEC total (mmol kg^{-1})	2.82
Sand content (%)	81.05
Silt content (%)	6.62
Clay content (%)	12.33

Source: Mazinagou *et al.* (2022a) [45]

Biological material

Four maize varieties were used as biological material: Ikenne 9449 SR (Ikenne); Tzee W pop STR QPM (Tzee), Sotubaka and Sammaz 52. The characteristics of these varieties were described in the national catalog of plant species and varieties grown in Togo (MAEP, 2013) [42]; the ECOWAS-UEMOA-CILSS catalogs of plant species and varieties (CORAF, 2017 and CORAF, 2019) [16; 17] and by Laba and Sogbedji (2015) [37]. The table below summarizes the characteristics of these varieties.

Table 2: Characteristics of varieties used for experimentation

Varieties	Genetic nature	Plant breeder	Maintainer	Height (cm)	Grain Colour	Production Cycle (Days)	Potential yield (T-ha^{-1})	Pest Resistance
Ikenne 9449 SR (Ikenne),	Composite	CIMMYT/ IITA	ITRA	190-210	White	90-100	5	Average
TZEE W pop STR QPM (Tzee)	Composite	IATI	ITRA	170-185	White	80-85	3.50	Average
Sotubaka	Composite	I	ITRA	210-230	Yellow	100-110	6	Average
Sammaz 52	Improved variety (Rich in Vitamin A)	Menkir Abebe	IAR	190-195	Orange	95 (average)	6	Non-tolerant

Sources: MAEP (2013) [42]; Laba and Sogbedji (2015) [37]; CORAF (2017) [16]; CORAF (2019) [17]

Fertilizers

The fertilizers used for the experiment consisted of complex fertilizer, NPK: 15-15-15, simple fertilizer, urea 46% N and ZinGap wettable powder fertilizer with 12% EDTA chelated Zn.

Table 3: Zinc rates applied and corresponding quantities of ZinGap with 12% of Zn and Zinc

Zinc content (%)	0 (Zn_0)	45 (Zn_{45})	60 (Zn_{60})
ZinGap quantity 12% Zn (kg ha^{-1})	0	3.75	5.00
Zinc quantity (g ha^{-1})	0	450	600

Experimental design

The experiment took place during the first growing seasons (April to August) of two consecutive years (2020 and 2021). Four maize varieties: Ikenne; Tzee; Sotubaka and Sammaz 52 and three zinc doses: control (0 kg ha^{-1}=Zn0); 3.75 kg ha^{-1} (Zn45) and 5 kg ha^{-1} of ZinGap at 12% Zn (Zn_{60}) were used. The combination of these two factors (varieties and zinc doses) gave twelve (12) treatments. The trial was set up using a split-plot design with three replications of each treatment. The main plots included the zinc doses and the sub-plots, the varieties. Nine (09) main plots (15 m x 3.20 m) and thirty-six (36) sub-plots (3.20 m x 3 m) were laid out. The distance between the blocks is equivalent to that between the plots and which was 1m. Each sub-plot contains five rows separated from each other by 0.80 m.

Soil and crop management

At the beginning of each cropping season, the experimental site was prepared through the following successive operations: Clearing, deep ploughing, levelling, and demarcation of blocks, main plots and subplots. The maize seeds were sown on April 14, 2020 and April 25, 2021 at four seeds per pocket, follow-up of thinning at one plant per pocket carried out ten (10) days after sowing. The planting pattern was 80 cm x 25 cm, giving a density of 50,000 plants ha^{-1}. 200 kg ha^{-1} of NPK: 15-15-15, were applied to the plants on the 15th day after sowing. 100 kg ha^{-1} of urea 46% N was applied at the beginning of flowering of each variety (table 4). NPK: 15-15-15 and urea 46% N were point-placed at a depth of approximately 5 cm. Zinc fertilizer (ZinGap with 12% of Zn) were brought by foliar application in two times (table 4). The water quantity used for each Zn spray was 350 litters ha^{-1}. Zinc foliar application was done between 3 p.m. and 5:30 p.m. Two weeding and one hilling were carried out respectively on the 14th, 30th and 45th day after sowing. Two insecticide treatments against the caterpillars were done and the crop was harvested on August 14, 2020 and August 21, 2021. The table below shows the application dates for Zin Gap.

Table 4: ZinGap application dates on plants of each variety

Product application dates	Ikenne	Tzee	Sotubaka	Sammaz 52
Dates of appearance of panicles (DAS)	45	38	55	45
Dates of first application of ZinGap (DAS)	46	39	56	46
Dates of second application of ZinGap (DAS)	60	53	70	60

DAS= Day after sowing

Data collection

Maize grains yield determination

Maize grain yields were determined from the three (03) centre rows of each subplot. The harvested cobs were dried, shelled and dried again. The maize grain weights were taken when the moisture content of the grains was around 12% (Mazinagou *et al.*, 2022b) [46].

Sampling

Maize cobs were harvested from nine (09) central plants in the three centre rows of each subplot (Mazinagou *et al.*, 2022a) [45]. The cobs were dried, shelled and dried again. Composite samples of maize grains of the same colour obtained after sorting were made. Samples of 50g of maize grains were taken from the seeds of each maize variety and from each composite sample of harvested maize grains. Similarly, composite samples of 50 g of soil were made and submitted to analysis. They were analysed in the Geochemistry and Environment Laboratory (LGE).

Chemical analysis methods

The maize grains samples were oven dried at 70°C for 48 h, then finely ground and sieved. Soil samples were also ground and sieved. Soil samples were also finely ground and sieved. The solubilization method used for soil samples was mineralization by acid attack (mixture of hydrochloric acid and nitric acid) in accordance with NF ISO 11466 or the aqua regia method (ISO, 1995) [30] for soils. It was carried out in a closed environment and at high temperature (110-150°C). For 1 g of soil sample finely ground and weighed on a BEL L303i electric balance (Pmax = 310 g, Accuracy = 0.001), a 3:1 ratio of acid mixture was required (3ml hydrochloric acid and 1ml nitric acid). For maize grains samples, the solubilization method used was mineralization by acid attack with nitric acid. It was also carried out in a closed environment and at high temperature (150°C). For 1 g of finely ground maize sample and weighed on a BEL L303i electric balance (Pmax=310 g, Accuracy= 0.001), 4 ml of nitric acid were required. 10 ml of 9% hydrogen peroxide (H2O2) was added beforehand to each sample and left to react for 24 hours before acid etching.

After acid etching and heating, the samples were filtered using filter paper (Walsh and Beaton, 1977) [61]. The filtrate obtained contained the chemical elements to be assayed.

Zinc contents in soil and maize grains were determined by atomic absorption spectrophotometry (Pinta, 1973; Tee *et al.*, 1989) [49, 59]. The brand name of the atomic absorption spectrophotometer used was ICE 3000 SERIES THERMO FISCHER.

Data analysis

The data collected were entered and processed using Excel spreadsheets. These data were tested for normality and were subjected to an analysis of variance (ANOVA) using GenSTAT software at the 5% threshold. Duncan's test was used to discriminate the means at this threshold

Results and Discussion

Influence of zinc doses on maize grain yield

Table 5 presents maize grain yields obtained under the different zinc doses. Zinc doses had a significant effect on maize grain yields. Yields obtained in the first year of experiment were 13.41% higher than those obtained in the second year. This superiority of first-year yields over second-year yields was due to climatic variability (Adewi *et al.*, 2010; Amouzou *et al.*, 2013) [1, 5]. Indeed, the cumulative rainfall obtained from April to August 2021 (540.90 mm) in the study area was higher than that recorded in 2020 (342.50 mm) at the same period. It therefore appeared that rainfall could influence the effectiveness of foliar zinc application on grain maize yield. In addition, the difference between the yields of the two years of experiment could be explained by the effect of previous crops and especially by the variation in the chemical composition of the soil (Mazinagou *et al.*, 2022a) [45]. These results were similar to those of Sogbedji *et al.* (2017) [56], who found a drop in yield ranging from 24 to 38% during the main cropping season of the second year compared to the first year, due to rainfall deficits; but in this study, it was the poor distribution of rainfall which led to lower maize grain yield during the second year of cultivation. Several studies (Lansigan *et al.*, 2000; Baulcombe *et al.*, 2009; Hatfield *et al.*, 2011) [38, 7, 26] had also proved that climate variability had direct impacts on crop production and consequently on food security and economic stability.

For the 2-year experiments, the highest yields were obtained with the foliar application of 5 kg ha^{-1} of ZinGap with 12% Zn (Zn_{60}). These yields were statistically identical to those recorded with 3.75 kg ha^{-1} of ZinGap at 12% Zn (Zn_{45}). However, the 2-year average yields obtained with Zn_{60} were 15.13 and 3.65% higher than those got with Zn_0 (control) and Zn_{45} respectively. The highest maize grain yields recorded under Zn_{60} would be due to the high amount of zinc supplied, which would have contributed to the growth and development of the maize plants. Zn acted as an essential component of many enzymes and controls several biochemical processes in plants necessary for growth (IRRI, 2000) [29]. These results showed that zinc application improved maize grain yield. Sudha and Stalin (2015) [58] had recorded lower paddy rice yields with low zinc concentrations when low amounts of zinc were applied. Increasing doses of Zinc had also a beneficial effect on strawberry production (Impa *et al.*, 2013) [28]. It was well documented that the application of Zn fertilizers not only increased yields, but also improved crop quality in wheat (Haslett *et al.*, 2001) [25] and rice (Li *et al.*, 2003) [39].

Effects of variety and zinc dose interactions on maize grain yields

Maize grain yields obtained under different variety and zinc dose interactions were registered in the table below. These yields ranged from 1.88±0.16 to 4.06±0.12 t ha^{-1} and from 1.86±0.06 to 3.55±0.13 t ha^{-1} respectively in the first and second years of experimentation. 2-year average yields ranged from 1.87±0.11 to 3.81±0.13 t-ha^{-1}. Statistical analysis showed that variety and zinc dose interactions significantly influenced maize grain yields. Maize grain yields in the first year of experimentation were higher than those of the second year. The yields decline in the second year was due to poor rainfall distribution in this year, because the amount of rainfall recorded the second year of experiment was greater than those of the first year. In the first and second years of experiments, the highest maize grain yields under the four varieties were obtained with foliar application of 5 kg ha^{-1} of ZinGap with 12% Zn (Zn_{60}); but the yields got with the Zn_{60} application to Ikenne and Sammaz 52 were statically identical to those of Zn_{45} under the same varieties in the first year of cultivation. In contrast, in the second year of experiment, the yields got with the application of Zn_{60} were statically similar to those obtained with Zn_{45} under all varieties. The results obtained in this study were similar to those of Keram *et al.* (2012) [33], who reported that treatments applied with increasing doses of Zn gave higher grain and straw yields than treatments applied with NPK alone. Indeed, for all four varieties, yields recorded under Zn_{60} were significantly higher than those obtained under Zn_0 (control with NPK applied) and Zn_{45}. Some authors (Akram *et al.*, 2020) [3] recorded the highest wheat grain yield (5.41 t ha^{-1}) with the application of 5 kg ha^{-1} of Zn. Zinc plays multiple roles in fundamental biochemical processes in plants, including enzyme activation, protein synthesis, starch, auxin and nucleic acid metabolism, and pollen development (Marschner, 1995; Cakmak, 2000; Chang *et al.*, 2005) [44, 10, 15].

For all the interactions, the highest yields of the 2-year experiments (4.06±0.12 t ha^{-1} in the first year and 3.55±0.13 t ha^{-1} in the second year) were observed under Sotubaka with the application of Zn_{60}. These highest yields obtained under this variety with Zn_{60} application could be explained by the intrinsic genetic characteristics of this variety, which enabled the plants to absorb sufficient quantities of the zinc applied. This superiority could also be due to the difference in potential yields, which could vary the nutrient requirements of plants. Indeed, the potential yields of the varieties were 5 t ha^{-1} for Ikenne; respectively, 3.50 t ha^{-1} for Tzee and 6 t ha^{-1} for Sotubaka and Sammaz 52. With this difference in potential yields, Ikenne and Tzee varieties would never be able to give a maize grain yield identical to that of sotubaka and Sammaz 52under normal conditions (especially good climatic conditions) of maize cultivation. The difference in yield observed between Sotubaka and Sammaz 52 would be linked to their production cycles (Mazinagou *et al.*, 2022b) [46]. Overall, the average yields obtained with Sotubaka are higher than those obtained with Ikenne, Tzee and Sammaz 52 by 26.88, 72.68 and 4.42% respectively. It indicated in Sudha and Stalin (2015) [58] study that grain and straw yields of different rice genotypes were significantly increased with zinc application by 14 and 16% respectively.

Table 5: Maize grain yields under variety and zinc dose interactions

Varieties	**Zinc doses**			**Means**	**F.Pr**	**CV (%)**
	Zn_0	**Zn_{45}**	**Zn_{60}**			
Maize grain yields (t ha^{-1})						
Year 1						
Ikenne	2.69±0.10b	3.02±0.10a	3.04±0.11a	2.92±0.39b	0,003	10,90
Tzee	1.88±0.16c	2.15±0.09b	2.43±0.10a	2.15±0.45c	<.001	12,70
Sotubaka	3.43±0.14c	3.87±0.10b	4.06±0.12a	3.78±0.50a	<.001	11,30
Sammaz 52	3.53±0.07b	3.71±0.09a	3.73±0.11a	3.66±0.36a	0,003	11,30
Means	2.88±0.71b	3.19±0.72a	3.32±0.73a	3.13±0.78	0,048	14,20
Year 2						
Ikenne	2.40±0.14b	2.78±0.12a	2.82±0.10a	2.67±0.45b	0,03	16,40
Tzee	1.86±0.06b	1.91±0.09ab	2.07±0.12a	1.94±0.35c	0,043	17,60
Sotubaka	2.93±0.13b	3.41±0.12a	3.55±0.13a	3.30±0.52a	0,003	14,20
Sammaz 52	2.95±0.23b	3.19±0.13a	3.23±0.12a	3.13±0.56a	0,011	12,30
Means	2.53±0.49b	2.82±0.62a	2.92±0.61a	2.76±0.69	0,027	17,00
2-year averages						
Ikenne	2.55±0.14b	2.90±0.10a	2.93±0.06a	2.79±0.40b	0,004	11,50
Tzee	1.87±0.11c	2.03±0.09b	2.25±0.10a	2.05±0.38c	<.001	13,40
Sotubaka	3.18±0.07c	3.64±0.11b	3.81±0.13a	3.54±0.51a	<.001	11,70
Sammaz 52	3.24±0.08b	3.45±0.09a	3.48±0.07a	3.39±0.35a	0,002	10,70
Means	2.71±0.60b	3.01±0.67a	3.12±0.66a	2.94±0.73	0,005	13,40

Zn_0= 0 kg ha^{-1}; Zn_{45} = 3.75 kg ha^{-1} of ZinGap at 12% Zn. and Zn_{60} = 5 kg ha^{-1} of ZinGap at 12% Zn; F.Pr = Fisher's probability; CV= Coefficient of variation. Data were discriminated horizontal direction. Values followed by the same letters are statistically identical.

Assessment of zinc dose performance under each variety

Table 6 shows the average performance rates of zinc doses in relation to the potential yields of varieties. The performance rate of a dose corresponds to the ratio between maize grain yield obtained after application of this dose and the potential yield of the variety to which this dose was applied. The highest performance rates were obtained under all varieties with the application of Zn_{60}. The effectiveness of this dose under all varieties could be explained by its ability to provide plants of different maize varieties with the nutrients they need for their growth and fruiting (Mazinagou *et al.*, 2022b) [46]. Tzee gave the highest performance rate (64.29%) with Zn_{60} application; although its yield is the lowest of the yields obtained with the application of Zn_{60} to the four varieties. Indeed, with the application of Zn_{60}, the two-year average yield obtained under Tzee is lower than those of Ikenne, Sotubaka and Sammaz 52 by 23.21; 40.94 and 35.34% respectively. On average basis, Sotubaka variety performed best. The highest performance of Sotubaka could be explained by its greater capacity to absorb more zinc than the other varieties. In addition, the production cycle and level of plant organ constitution of each variety (Maltais, 2006; Mazinagou *et al.*, 2022a) [43, 45] could also explain the difference in performance between varieties after dosing. With regard to the performance rates of zinc doses in relation to yield and from an economic view point, Zn_{60} application would have further improved the profitability of maize production with Tzee and Sotubaka varieties; while the best profitability of maize production with Ikenne and Sammaz 52 varieties could be obtained with Zn_{45} application.

Table 6: 2-year averages of performance rates of zinc doses under each variety

Varieties	**Zinc Doses**			**Means**
	Zn_0	**Zn_{45}**	**Zn_{60}**	
Performance rate (%)				
Ikenne	51,00	58,00	58,60	55,80
Tzee	53,43	58,00	64,29	58,57
Sotubaka	53,00	60,67	63,50	59,00
Sammaz 52	54,00	57,50	58,00	56,50

Zn_0= 0 kg ha^{-1}; Zn_{45} = 3.75 kg ha^{-1} of ZinGap at 12% Zn. and Zn_{60} = 5 kg ha^{-1} of ZinGap at 12% Zn

Assessment of zinc uptake and accumulation in maize grains

Grain zinc concentrations obtained after chemical analysis of maize grains in the laboratory were recorded in table 7. Zn concentrations in the second year of experiment were 8.24% higher than those of the first year. The results showed that maize varieties absorbed and accumulated zinc in their grains in different ways. In the first year of experiment, the highest zinc concentration was obtained in the Sammaz 52 grains (53.47±3.08 mg kg^{-1}), while it was higher in Sotubaka grains (60.07±3.83 mg kg^{-1}) in the second year. Based on the 2-year averages of zinc concentration in maize grains, the highest zinc concentration was observed in Sotubaka grains (55.62±3.78 mg kg^{-1}). This concentration was higher than those of Ikenne, Tzee and Sammaz 52 grains by 17.02; 7.13 and 2.64% respectively. This difference in grain zinc accumulation among the varieties was due to genetic characteristics and the nature of plant organs especially stomata, trichomes, cuticle, cuticular and epicuticular wax and cutin which influenced the efficiency of foliar fertilization (Maltais, 2006) [43]. Other factors such as: Soil, climate, plants and their interaction also affected nutrient uptake by plants (Fageria *et al.*, 2009) [20]. According to de Valença *et al.* (2017) [18], the bioavailability of micronutrients from the crop to the food was influenced by the crop (variety), which defines whether micronutrients are (re-)localized in the edible parts of the crop.

Table 7: Zinc concentrations in maize grain of each variety

Years	**Varieties**				**Means**	**F.Pr**	**CV (%)**
	Ikenne	**Tzee**	**Sotubaka**	**Sammaz 52**			
Zinc concentrations (mg kg^{-1})							
Year 1 (2020)	46.27±2.68c	50.09±2.87b	51.17±3.02b	53.47±3.08a	50.25±4.19	< 0.001	6,90
Year 2 (2021)	48.80±3.42c	53.76±3.68b	60.07±3.83b	54.92±3.66a	54.39±4.53	< 0.001	9,50
Means	47.53±3.05c	51.92±3.38b	55.62±3.78a	54.19±3.46a	52.32±4.36	< 0.001	7,90

F.Pr = Fisher's probability; CV= Coefficient of Variation. Data were discriminated horizontal direction. Values followed by the same letters are statistically identical

Effect of zinc doses on zinc concentration in maize grains

Table 8 shows the grain zinc concentrations recorded under each zinc dose. The results indicated that zinc doses also had a significant effect on zinc concentration in maize grains. The highest grain zinc concentrations were obtained with the foliar application of 5 kg ha^{-1} of ZinGap with 12% Zn (Zn_{60}) in first (61.98±2.46 mg kg^{-1}) and second year (70.19±2.79 mg kg^{-1}) of experiment. On 2-year average basis, grain zinc concentration recorded under Zn_{60} (66.09±2.31 mg kg^{-1}) were higher than those of Zn_0 and Zn_{45} by 118.12 and 9.10% respectively. Some authors showed that zinc increasing doses supplying to rice plants increased the total zinc content per plant at different stages of strawberry growth (Sarwar *et al.*, 2013) [54]. The effectiveness of this dose (Zn_{60}) would be linked to the quantity of zinc supplied (5 kg ha^{-1} for Zn_{60} versus 3.75 kg ha^{-1} for Zn_{45}), which would have allowed the plants to absorb sufficient zinc and increase its accumulation in the grains, regardless of climatic or environmental conditions which could influence its uptake by the plants. Indeed, relative humidity, light intensity, rain and wind could affect the efficacy of a foliar fertilization at the time of, or just after foliar application of the product (Maltais, 2006) [43]. Some authors (Sanjeeva *et al.*, 2020; Chandel *et al.*, 2010) [52, 14] showed that Zn concentration in grain depends on environmental factors such as temperature, soil type, soil pH and micronutrient availability in the soil. However, zinc application increased zinc concentration in maize grain. It proved in some studies that the foliar application of Zn after flowering had been effective in increasing zinc content in rice grains (Boonchuay *et al.*, 2013 and Yuan *et al.*, 2013) [8; 66]. In this study, the foliar application of zinc was made at two stages, early flowering and two weeks after the first application; but the results obtained lead to the conclusion of these authors. According to Phattarakul *et al.* (2012) [48] and Jalal *et al.* (2020) [31], the timing of application (crop stage) and the number of applications were imperative to increase Zn accumulation in the grain (Peramaiyan *et al.*, 2022) [47].

Table 8: Grain zinc concentrations under zinc doses

Years	**Doses de Zinc**			**Average**	**F,Pr**	**CV (%)**
	Zn_0	**Zn_{45}**	**Zn_{60}**			
Zinc concentrations (mg kg^{-1})						
Year 1 (2020)	31.68±2.13c	57.09±2.23b	61.98±2.46a	50.25±4.19	<.001	6,90
Year 2 (2021)	28.91±2.91c	64.06±2.86b	70.19±2.79a	54.39±4.53	<.001	9,50
Averages	30.30±2.01c	60.58±2.29b	66.09±2.31a	52.32±4.36	0,001	8,90

Zn_0= 0 kg ha^{-1}; Zn_{45} = 3.75 kg ha^{-1} of ZinGap at 12% Zn. and Zn_{60} = 5 kg ha^{-1} of ZinGap at 12% Zn

F.Pr = Fisher's probability; CV= Coefficient of Variation. Data were discriminated horizontal direction. Values followed by the same letters are statistically identical

Influence of variety and zinc dose interactions on zinc concentration in maize grains

Grain zinc concentrations obtained under variety and zinc dose interactions ranged from 29.17±2.51 to 66.22±2.96 mg kg^{-1} and from 26.97±2.98 to 78.23±2.88 mg kg^{-1} respectively in first and second year of experiments (table 9). Overall, grain zinc concentrations recorded in second year of experiment were higher than those of the first year. This difference could be due to climatic or environmental conditions, which could have a negative impact on the efficient use of zinc supplied by maize plants in the first year. Indeed, cumulative rainfall from April to August 2021 (540.90 mm) in the study area was higher than that recorded in 2020 (342.50 mm) over the same period. According to Maltais (2006) [43] and Mazinagou *et al.* (2022a) [45], relative humidity, light intensity, rain and wind can affect the efficacy of product's foliar application at the time of or just after its foliar application.

For the two experiments, the highest grain zinc concentrations under the four varieties were obtained with the application of 5 kg ha^{-1} of ZinGap with 12% Zn (Zn_{60}); but the best grain zinc concentrations were obtained in the first year under Sammaz 52 (66.22±2.96 mg kg^{-1}) and in the second year under Sotubaka (78.23±2.88 mg kg^{-1}). These results were similar to those of Khampuang *et al.* (2020) [34], who proved that foliar Zn application could improve grain Zn concentration; but the response could change according to cropping year and cultivar or variety. On 2-year average basis, Zn_{60} foliar application to the Sotubaka (70.58±2.91 mg kg^{-1}) gave the highest grain Zn concentration. The highest concentration observed under this interaction could be due to the performance of Sotubaka variety in terms of zinc uptake and accumulation, and the effectiveness of this dose to provide sufficient zinc amount to maize plants for their growth and fruiting. Indeed, zinc plays multiple roles in fundamental biochemical processes in plants, including enzyme activation, protein synthesis, starch, auxin and nucleic acid metabolism, and pollen development (Chang *et al.*, 2005) [15]. Some authors (Khampuang *et al.*, 2022) [35] found that Zn concentration in the grains of the CNT1 variety increased from 19.5% to 32.6% compared with the control (without Zn), when rice plants received Zn by foliar application. Veni *et al.* (2019) [60] also observed the highest Zn contents in rice grain (21.20 mg kg^{-1}) and straw (33.20 mg kg^{-1}) in CSR-30 cultivar. The bioavailability of micronutrients including zinc therefore depends on numerous soil factors and the crop or variety (de Valença *et al.*, 2017) [18]. This conclusion is obvious, as there was a difference in zinc uptake and accumulation in the grains of maize varieties in this study. Maize varieties, soil and climatic factors were therefore determinants of zinc accumulation in maize grains.

Table 9: Zinc concentrations in maize grains under variety and zinc dose interactions

Varieties	**Doses de Zinc**			**F,Pr**	**CV (%)**
	Zn_0	**Zn_{45}**	**Zn_{60}**		
Zinc concentrations (mg kg^{-1})					
Year 1 (2020)					
Ikenne	29.17±2.51c	52.76±2.88b	56.88±2.68a	0,001	7,30
Tzee	31.82±2.79c	56.55±2.74b	61.91±2.94a	<.001	6,80
Sotubaka	31.85±2.88c	58.74±2.89b	62.93±2.95a	<.001	6,90
Sammaz 52	33.89±2.92c	60.30±2.62b	66.22±2.96a	<.001	7,00
Year 2 (2021)					
Ikenne	26.97±2.98c	56.80±2.94b	62.63±2.99a	<.001	7,10
Tzee	29.02±2.84c	62.74±2.86b	69.51±2.87a	<.001	6,70
Sotubaka	29.09±2.73c	72.90±2.96b	78.23±2.88a	<.001	6,60
Sammaz 52	30.56±2.95c	63.79±2.96b	70.41±2.91a	<.001	6,80
2- year averages					
Ikenne	28.07±2.64c	54.78±2.63b	59.75±2.84a	0,002	7,40
Tzee	30.42±2.82c	59.65±2.65b	65.71±2.90a	0,003	8,10
Sotubaka	30.47±2.81c	65.82±2.92b	70.58±2.91a	<.001	7,00
Sammaz 52	32.22±2.94c	62.05±2.61b	68.31±2.93a	<.001	7,40

Zn0= 0 kg ha^{-1}; Zn45= 3.75 kg ha^{-1} of ZinGap at 12% Zn. and Zn60= 5 kg ha^{-1} of ZinGap at 12% Zn.
F.Pr = Fisher's probability; CV= Coefficient of variation. Data were discriminated in the horizontal direction. Values followed by the same letters are statistically identical.

Conclusion

At the end of this study aimed at improving yield and zinc concentration in maize grains through agronomic biofortification, it was found that zinc foliar application considerably improved yield and zinc concentration in maize grains. The 5 kg ha^{-1} of ZinGap with 12% Zn (Zn_{60}) increased both maize grain yield and grain zinc concentration. The Sotubaka variety accumulated more zinc in its grains than Sammaz 52, Tzee and Ikenne varieties. Zn_{60} foliaar application to varieties gave both the highest yield and zinc concentration under each variety. However, it would be necessary to carry out an economic profitability study in order to make appropriate recommendations. It would also be important to enhance the availability and accessibility of zinc fertilizers to increase their use by the producers.

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