World Journal of Agricultural Sciences 9 (3): 290-297, 2013
ISSN 1817-3047

DOI: 10.5829/idosi.wjas.2013.9.3.1741

Fungicides and Essential Oils for Controlling Maize Seed-Borne *Fusarium moniliforme* and its Transmission into Seedlings

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Abstract: Seed borne diseases are important constraints to maize production. Investigations were carried out to identify fungal infection of maize seed and determine its effect on seed germination. *Studies* on the effects of fungicides and essential oils from aromatic plants on the level of seed-borne *F. moniliforme* and its seed to seedling transmission were also part of the work. Blotter method was used for seed health testing while germination in sand was followed for seed germination test. Maize seed samples identified with low infection (1-10%), medium infection (11-30%) and heavy infection (<30%) of *F. moniliforme* showed a germination of 93%, 98% and 85% respectively proving that heavy infection of this fungus reduces significantly the germination and should be controlled. Seed treatment with 3 fungicides i.e Apron star, Benlate and Marshall resulted into a significant increase in the germination only in the heavily infected category. It could be concluded that seed lot identified as low and medium *F. moniliforme* infection can be sown without any seed treatment. The control of *F. moniliforme* in seeds was achieved at 90% in all the 4 cultivars tested using the essential oils extracted from *O. gratissimum* and *C. citratus.* Improvement of the germination was recorded from the treatment with the essential oils. The control of seed to seedling transmission of *F. moniliforme* was achieved at 47% for the essential oils *O. gratissimum* and 31% for *C. citratus*. These control effects of the essential oils from *O. gratissimum* and *C. citratus* on *F. moniliforme* in maize seeds as well as the improvement in the germination disclosed the potential use of the 2 essential oils studied as seed treatment against fungi in maize.

Key words: Esential oil · Maize · Seed-borne · Fusarium moniliforme · Control

INTRODUCTION

Ear rot of maize is caused by many fungi among which *F. moniliforme* is the most important. From previous studies conducted in Denmark from 1993-1995, this fungus was found prevalent in maize seed samples of Cameroon with heavy infection of up to 80% in the tested samples [1]. This level of infection of *F. moniliforme* has also been recorded in other samples tested from 1995-2000 [2]. The fungus is seed-borne and seed transmitted with a transmission rate of 1:1 [3, 1]. Systemic infection from seed-borne inoculums has been demonstrated [4, 5]. The systemic infection of *F. moniliforme* results into symptomless plants, the reestablishment of the fungus in the kernel and mycotoxins accumulation during its development. Others effects of *F. moniliforme* are loss of production in the field, post harvest losses, mycotoxins in grains and in the subsequent crop, loss of germination, seedling blight, stalk and ear rot disease [5-10]. These effects have been studied on seed, at seedling level and plant tissues. However correlations between different levels of natural infection of *F. moniliforme* in seeds and their effects on the germination and germination components, emergence of seedlings is not

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well understood. Antifungal and antimicrobial properties of essential oils have been demonstrated by [11-15]. Despite this knowledge the use of these essential oils as seed treatment to control the seed-borne *F. moniliforme* and its seed to seedling transmission had been very limited. This study was planned to investigate different levels of seed borne infections of *F. moniliforme* and their effect on seed germination and possibility of seed to seedling transmission.

Studies on the effects of fungicides and essential oils from aromatic plants on the infection of this seed-borne fungus and its seed to seedling transmission was another objective of this work..

MATERIALS AND METHODS

Effects of Low, Medium and High Infections of *F. Moniliforme* on the Germination of Maize Seeds and Their Control Using Fungicides: Sixteen maize seeds samples registered with DGISP accession number were used. They were selected based on their level of *F. moniliforme* infection recorded during seed health testing, 8 samples with high (above 30%) level of infection, 5 samples with medium (11-30%) infection and 3 samples with low (1-10%) infection (Table 1). Three fungicides were used, Apron Star promoted in Cameroon for seed treatment by Novartis international, Benlate (benomyl) manufactured by Dupont France and Marshall (carbofuran) distributed to farmers in Cameroon by Sodecoton.

A total of 200 seeds in 4 replicates of 50 seeds were tested from each samples and for each of the 4 treatments defined. The treatment was performed by dusting at the dosage of 0.1% (w/w) [16, 17] and 2 drops of water was mixed with the seeds before addition of fungicides based on the suggestion of Novartis International. The germination test was performed in 4 replicates of 50 seeds following the sand method described by [18].

Screening of Essential Oil Rate of Application Efficient Against *F. Moniliforme* and Harmless to Maize Seeds Germination Capacity: Maize samples cultivars CMS 8704, registered with DGISP number 43903 and 44443 were used. They were both identified with 100% infection of *F. moniliforme* during seed health testing. Two essential oils extracted from the aromatic plant *Ocimum gratissimum* and *Cymbopogon citratus* by hydro-distillation with the Clevenger apparatus were used to treat the seeds [12]. Three concentrations of each essential oil, 10%, 7% and 5% for *O. gratissimum*

Table 1: Effects of low, medium and high infections of *F. moniliforme* on the germination of maize seeds and their control using 3 fungicides (Apron star, Benlate and Marshall).

			Germination			
No	DGISP number	Infection *F.moniliforme*	Non treated	Apron star	Benlate	Marshall
1	43910	2%	99%	100%	100%	-
2	44138	5%	95%	93%	94%	92%
3	44192	5%	84%	83%	78.5%	83%
mean 1	4%	[c]93%	92%	91%	87.5%	
4	43902	14%	99%	100%	100%	-
5	43975	14%	97%	99%	99.3%	99
6	44151	14%	97%	98.5%	99%	98
7	44172	23%	99%	100%	100%	100%
8	43977	24%	97.5%	98%	98%	100%
mean 2	17.8%	[b]98%	99.10%	99.26%	99.25%	
9	43994	33%	83%	91%	87%	90%
10	44136	54%	54%	60%	58%	58%
11	44189	54%	94%	98%	99%	98%
12	44145	59%	74%	84%	78%	87%
13	43905	73%	94%	97%	97.5%	96%
14	43985	73%	87.5%	91%	91%	-
15	43904	96%	97%	97%	100%	-
16	43912	100%	92.6%	99%	91%	-
mean 3	68%	[a]85%	90%*	88%	86%	

(-) Not tested due to sample size

The numbers in the table are means of 4 replications of 50 seeds

(*)Denote statistical differences between the germination percentage in the row (α= 0.05)

Different letter denote statistical difference between the germination of infected seeds in the column (α =0.05)

and 3%, 2% and 1% for *C. citratus* were screened for their control effect on *F. moniliforme* in seeds and inhibitor effect on the germination of the treated maize seeds. Efficient and harmless concentrations were selected for subsequent seed treatment. The essential oil was suspended in an agar-based solution following [19] and the treatments were carried out at the ratio of 50µl/g of seeds, modified by [20]. The germination test was carried out as described above. One hundred seeds were tested for each essential oil and each concentration. The harmful effect was determined as reduction in germination in the treated seed compared to none treated.

Control of *F. Moniliforme* Infections in Maize Using the Essential Oil from *O. Gratissimum* and *C. Citratus*: Four maize seed samples of different cultivars were used, CMS 9015, CMS 8806, CMS 8704 and CMS 8501. The samples were registered with DGISP number 44445, 44444, 44443, 44440 and identified with 60%, 70% 100% and 76% infection of *F. moniliforme* respectively. Four treatment were evaluated, control (untreated), positive control (treated with benlate), treatment with the essential oil from *O. gratissimum* and treatment with essential oil from *C. citratus*. The Treatment with the Essential Oil Was Performed atThe ratio is 2% for *C. citratus* and 6% for *O. gratissimum*. The essential oil carrier was prepared from Agar following [19]. The Benlate fungicide was applied as described above. After treatment, the sample was divided into two groups one for the seed health testing and another for sowing. The treated seeds were tested in 4 replications of 50 seeds for *F. moniliforme* using the deep freezing blotter method of [21] and the experiments were repeated twice. The infection was the mean percentage of infected seeds over the total number of tested seeds.

Control of Seed to Seedling Transmission of *F. Moniliforme* in Maize Using the Essential Oil from *O. Gratissimum* and *C. Citratus*: The fraction of seeds maintained from previous section from any of the four cultivars and treatments was germinated in sterile sand, following the Rules of the International Seed Testing Association (ISTA). Two hundred seeds in 4 replicates of 50 seeds were tested in each treatment and for each cultivars and the experiment was repeated twice. Seedling evaluation was performed after 10 days. Data on germination were taken and the seedlings were assay for *F. moniliforme* following the method described by [1] in 3 replicates of 25 seedlings. The reduction in the seed to seedling transmission of *F.moniliforme* was calculated as the percent difference between the treated and untreated.

Data Analysis: The data were subjected to statistical analysis to determine the significance of differences using Sigmat Stat at 97% level of confidence [22].

RESULTS

Effects of Low, Medium and High Infections of *F. Moniliforme* on the Germination of Maize Seeds and Their Control Using 3 Fungicides

Effect of Low Medium and High Infection of *F. Moniliforme* on the Germination: Data in Table 1 showed that in untreated seeds, 93% germination was recorded in the less infected samples (1-10%), 98% in the medium infected (11-30%) and 85% in the heavily infected (>30%) samples. A difference of 5% was noted between the germination of less infected samples compared to the medium infected samples, the former having germinated less and the later having germinated higher. This difference however was not statistically significant at 95% level of confidence. Between the less infected samples and the heavily infected samples, a difference of 8% was noted, the less infected having germinated higher and this was statistically significant at 95% level of confidence. The medium infected and the heavily infected showed a difference of 13% germination, the first having germinated higher and this was statistically significant at 95% level of confidence.

Effects of Fungicides Treatment on the Germination of Low Medium and Highly Infected Seeds: After the treatment of less infected seeds with 3 different fungicides Apron, Benlate and Marshall, no improvement of the germination was recorded (Table 1). With the medium infected seed samples, the same 3 treatments resulted in a small improvement of about 1.5% in the germination. With the heavily infected samples, improvement of 1% was recorded from Marshall treatment, 3% from Benlate and 5% from Apron star. The improvement from Apron star was statistically significant at 95% level of confidence.

Screening of Essential Oil Rate of Application Efficient Against *F. Moniliforme* and Harmless to Maize Seeds Germinative Capacity: Seed treatment with the essential oil from *O. gratissimum* at the rate of 10% eradicated *F. moniliforme* but also inhibited the seeds by increasing the number of dead seeds from 7.5% in the untreated

Table 2: Efficiency of essential oil against *F. moniliforme* and their effect on maize seeds viability

Treatment	Normal plants	abnormal	Dead seeds	Germination %	*F. moniliforme* in seeds	Control
Non treated	92.5	0	7.5	92.5	100%	0
Og 5%	92.5	2.5	5	92.5	25%	75%
Og 7.5%	62.5	2.5	35	62.5	5%	95%
Og 10%	27.5	2.5	70	27.5	0%	100%
Cc 3%	97.5	2.5	0	97.5	7.5%	92%
Cc 2%	95	0	5	95	5%	95%
Cc 1.5%	95	2.5	2.5	95	2.5%	97%

Og = *Ocimum gratissimum* CC = *Cymbogon citratus*

Table 3: Control of *Fusarium moniliforme* in maize seeds treated with Benlate systemic fungicide and 2 essential oils.

Maize variety	Benlate (0.1%w/w)	*O.gratissimum* (6%)	*C.citratus* (2%)	Non treated control
CMS 8501	0	5	7	85
CMS 8704	3	12	6	76
CMS 9015	0	8	10	67
CMS 8806	0	3	6	56
Mean	0.75±1.5	7 ±4	7.25±2	71±12
control of seed-borne infection	99[a]	90[b]	90[b]	0[c]

The numbers in the table are per cent infected seeds recorded after 7 days incubation. The experiment was repeated 2 times. Different letter denote significant difference.

Table 4: Improvement of the germination of maize seed treated with the essential oil from *O. gratissimum* and *C. citratus*

Maize variety	Benlate (0.1%w/w)	*O.gratissimum* (6%)	*C.citratus* (2%)	Non treated control
CMS 8501	95	93	93	87
CMS 8704	94	95	93	90
CMS 9015	93	91	92	87
CMS 8806	95	90	94	90
Mean	94.25	92.25	93	88.5

The numbers in the table are per cent germination recorded from the second count after 7 days. They are mean of 4 replications of 50 seeds and the experiment was repeated 2 times.

seeds to 70% (Table 2). At the rate of 7.5%, 95% control of the fungus was obtained with an increase of 27.5% in the number of dead seeds compared to the non-treated. The same treatment at the rate of 5% provided 75% control of the fungus and 92.5% germination of the seeds. With the essential oil from *C. citratus* applied at the rate of 1.5%, 2% and 3%, a control of 97%, 95 and 92% was recorded respectively (Table 2). No phytotoxicity of the oils to the seeds was expressed at these concentrations.

Control of *F. Moniliforme* Infections in Maize Seeds Using the Essential Oil from *O. Gratissimum* and *C. Citratus*: The data in Table 3 shows a mean control of 90% infection of *F. moniliforme* in all the 4 cultivars (CMS 8501, CMS 8704, CMS 9015 and CMS8806) tested by the essential oils extracted from *O. gratissimum* and *C. citratus* whereas Benlate control 99%. These control effects of the two essential oils were statistically significant compare to the non-treated control, lower and statistically different compared to Benlate. Differences were observed between the responses of the 4 cultivars used, however they were not statistically significant (Table 3).

Improvement of the Germination of Maize Seed Treated with the Essential Oils from *O. Gratissimum* and *C. Citratus:* Different levels of improvement of the germination were recorded between the 4 cultivars tested as a result of treatment with the essential oils (Table 4). The mean germination obtained for each treatment was 92.25% for the essential oils from *O. gratissimum*, 93% for *C. citratus*, 88.5% for the untreated control and 94.25% for the control treated with Benlate. These were not statistically different.

In addition to the increase in the germination percent, the treatment with the essential oil improve the health of the seedling in term of greenness, vigour and root mass based on visual observation.

Table 5: Control of seed to seedling transmission of *Fusarium moniliforme* in maize seedlings after treatment with Benlate systemic fungicide and 2 essential oils.

Maize variety	Benlate (0.1%w/w)	*O.gratissimum* (6%)	*C.citratus* (2%)	Non treated control
CMS 8501	2±2.8	48±14	60±13	100±0
CMS 8704	6±3.7	53±36	74±16	98±2
CMS 9015	3±3.0	56±22	76±23	100±0
CMS 8806	0.8±2.0	50±18	63±38	95±4
Mean	3±2.0	52±3.5	68±8	98±2
control of seed to seedling transmission	97[a]	47[c]	31[c]	0[e]

The numbers in the table are per cent seedlings recorded with the growth of Fusarium moniliforme after 7 days incubation. They are means of 3 replications of 25 seedlings and the experiment was repeated 2 times. Different letter denote significant difference

Control of Seed to Seedling Transmission of *F. Moniliforme* in Maize Using the *O. Gratissimum* and *C. Citratus:* Different levels of control of seed to seedling transmission of *F. moniliforme* were recorded between the 4 cultivars tested as a result of treatment with the essential oils (Table 5). The mean control level recorded for each treatment was 47% for the essential oils from *O. gratissimum*, 31% for *C. citratus* and 97% for Benlate compared to the untreated control (Table 5). These control effect were statistically significant ($P<0.01$).

DISCUSSION

Maize seed samples identified with low infection (1-10%), medium infection (11-30%) and heavy infection (<30%) showed a germination of 93%, 98% and 85% respectively (Table 1). The difference between the germination of less infected, medium infected and the heavily infected categories were statistically significant at 95% level of confidence. The germination of the heavily infected seed samples was also low compared to the standard for germination of the International Seed Testing Association [23]. This showed that heavy infection of *F. moniliforme* affects significantly the germination and should be control. Although many studies have earlier focussed on the effects of *F. moniliforme* on seed, seedling, plant and plants tissues [5-10]. The effects of different level of this pathogen on the germination of seed lots were not well understood. These effects have been clearly demonstrated in the present work and this strongly underline that that speculations earlier made by [7, 10, 24] are not valid. Consequently high attention should be given to seeds lots having heavy infection in the samples tested.

Seed treatment with fungicides, Apron star, Benlate and Marshall did not produced any significant increase in the germination of low and medium infected seeds whereas the same treatments produced important increase in the germination of heavily infected seeds with the effect of Apron star statistically significant (Table 1). It could be concluded that, in cases where only the germination is considered, seed lots identified with low and medium infection of *F. moniliforme* can be sown without any treatment and seed lots with heavy infection sown only after seed treatment.

Although treatment of maize seed have been investigated and documented by [16, 25, 1,17] the type of speculations investigated here were not achieved before and this result is a scientific information that could support organic agriculture.

In some individual seed samples, increase from the treatment improves the standard of the seeds from the unacceptable standard to the acceptable according to the International Seed Testing Association [23].

In the present study the screening of application ratio of the essential oil from *O. gratissimum* shows that this essential oil should be applied within the limit of 5% and less than 7.5% of the oil carrier to control *F. moniliforme* and maintained high viability of maize seeds. The essential oil from *C. citratus* could be used without any inhibition of the germination at the rate of 1.5%-3% to achieve a good control *F. moniliforme* in seeds. Similar effect on the viability of maize seeds was recorded earlier by [14] after using a ratio of 10% of Dimethyl sufoxide to treat maize seeds. The use of appropriate ratio to apply the essential oil is a key factor to obtain a good germination and a better control of *F. moniliforme* in maize seeds. The ratio of 6% and 2% for *O. gratissimum* and *C. citratus* respectively were selected and used in the subsequent. The negative effect of higher concentration of essential on the germination has also been recorded by [26]. These authors noted that thyme oil used for onion seeds treatment at concentration higher than 0.25% adversely affect the germination.

Furthermore a mean control of 90% *F. moniliforme* was observed in all of the 4 cultivars (CMS 8501, CMS 8704, CMS 9015 and CMS8806) treated with the essential oils extracted from *O. gratissimum* and *C. citratus* whereas Benlate control 99%. These control effects of the two essential oils were statistically significant compare to the non treated control, lower and statistically different compared to Benlate. Although earlier investigations of [14] demonstrated the eradication of *F. moniliforme* in maize seeds using the essential oil extracted from *O. gratissimum,* the treated seed were not viable and the authors in their subsequent investigations focused more on the control of fungi and the limitation of mycotoxins contamination [15]. Study on the effect of essential oil from D*acus carota* on seed-borne *Aspergillus flavus* in Guar seeds were investigated by [27]. The essential oil from *C. citratus* has been tested against *Aspergillus flavus*, *Macrophomina phaseolina* and *Pseudomonas* sp. by [20]. Other essential oils were investigated against *Botrytis cinearea* by [28]. In the present study, the control of *F. moniliforme* using the essential oil from *O. gratissimum* and *C. citratus* are integrated in the perspective of using the essential oils as seed treatment and this has earlier been attempted by [29, 26]. These authors studied the effect of different concentrations of thyme oil against seed-borne bacteria and fungi of cabbage seeds.

On the other hand different levels of improvement of the germination were recorded between the 4 cultivars tested as a result of treatment with the essential oils (Table 4). The mean germination obtained for each treatment was 92.25% for the essential oils from *O. gratissimum*, 93% for *C. citratus*, 88.5% for the untreated control and 94.25% for the control treated with Benlate. In addition to the increase in the germination percent, the treatment with the essential oil improve the health of the seedling in term of greenness, vigour and root mass based on visual observation.

Such observations were not made before and this lead to thought that the essential oils from *O. gratissimum* and *C. citratus* have a very positive effect on the germination of maize as well as its development. This illustrate the potential used of the 2 essential oils to improve the standard of seed lots and also stressed the need of further investigation to improve their application and understand their action on the physiology of maize plant. Improvement of the germination of rice seeds using essential oils as seeds treatment was also achieved by [30].

Moreover; the treatment with the essential oils controlled the seed to seedling transmission of *F. moniliforme* in all the 4 cultivars tested (Table 5). The mean control level recorded for each treatment was 47% for the essential oils from *O. gratissimum* and 31% for *C. citratus*. These control from the essential oils although lower compared to Benlate were statistically significant compared to the untreated control ($P<0.01$). This result is in the same line with that of [30] who recorded an improvement of 4-8% germination in rice seeds after treatment with the essential oils from *O. gratissimum* and *C. Citratus.* These results, elucidates again the potential use of the 2 essential oils studied as seed treatment against *F. moniliforme* in maize seeds. The need of further investigation to better understand the mechanism and also determine better application method is stressed. This is in the same line with the recommendation of [26]. These authors mentioned very clearly that many governments should support research on organic seed treatments and underlined the need of collaboration between private and public institutions both on the national and international level.

CONCLUSION

Seed treatment with 3 fungicides, Apron star, Benlate and Marshall resulted in a significant increase in the germination only in the heavily infected seeds. Referring to these results it could be concluded that considering the germination, seed lot identified with low and medium infection of *F. moniliforme* can be sown without any treatment. The control of *F. moniliforme* in seeds was achieved in all the 4 cultivars tested using the essential oils extracted from *O. gratissimum* and *C. citratus.* It could be concluded that seed treatment is not cultivar dependent. Improvement of the germination was recorded from the treatment with the essential oils along with the control of seed to seedling transmission of *F. moniliforme*. These control effects of the essential oils in maize seeds as well as the improvement in the germination disclosed the potential use of the 2 essential oils studied as seed treatment against fungi in maize. Such essential oils are potential bioactive compound useful for seed treatment.

ACKNOWLEDGEMENT

This research was funding by DANIDA through the Danish Government Institute of Seed Pathology for developing Countries and The Cereals Program of the Institute of Agricultural Research for Development, Cameroon.

REFERENCES

1. Tagne, A., 1995. Acremonium strictum and Fusarium moniliforme in maize seed samples of Cameroon: Infection Biology Seed Infection and Treatment. MSc thesis, The Royal Veterinary and Agricultural University Copenhagen, Denmark, pp: 125.
2. Mathur, S.B., H.K. Manandhar, 2003. Fungi in Seeds, recorded at the Danish Government Institute of Seed Pathology for Developing Countries. Danish Government Institute of Seed Pathology for Developing Countries (DGISP), Copenhagen, Denmark.
3. Richardson, M.J., 1990. An Annotated List of Seed-borne Diseases. The International Seed Testing Association, Zurich, Switzerland, pp: 190.
4. Foley, D.C., 1962. Systemic infection of corn by Fusarium moniliforme Phytopathology, 52: 870-872.
5. Munkvold, G.P., D.C. McGee and W.M. Carlton, 1997. Importance of different pathways for kernel infection by Fusarium moniliforme. Phytopathology 87: 209-217.
6. Melchers, L.E. and C.O. Johnston, 1924. Second progress report on the studies of corn germination and the prevalence of Fusarium moniliforme and Diplodia zeae. Phytopathology, 14: 45.
7. Futrell, M.C. and M. Kilgore, 1969. Poor stands of corn and reduction of root growth caused by Fusarium moniliforme. Plant Disease Reporter, 53: 213-215.
8. King, S.B. and G.E. Scott, 1981. Genotypic differences in maize to kernel infection by Fusarium moniliforme. Phytopathology, 71: 1245-1247.
9. Kulik, M.M. and J.F. Schoen, 1982. Germination, vigor and field emergence of sweet corn seeds infected by Fusarium moniliforme. Seed Science and Technology 10: 595-604.
10. Styer, R.C. and D.J. Cantliffe, 1984. Infection of 2 endosperm mutants of sweet corn by Fusarium moniliforme and its' effect on seedling vigor. Phytopathologym, 74: 189194.
11. Awuah, R.T, 1989. Fungitoxic effects of extracts from some West African plants. Annals of Applied Biology, 115: 451-453.
12. Amwam Zollo, P.H., L. Biyiti, F. Tchoumbougnang, C. Menut and Ph. Bouchet, 1998. Aromatic plants of Central Africa. Part XXXII, Chemical composition and antifungal activity of thirtheen essential oils from aromatic plants of Cameroon. Flavour and Fragrance 13: 107-114.
13. Mishra, A.K. and N.K. Dubey, 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. Applied Environmental Microbiology, 60: 1101-1105.
14. Tagne, A., J. Nguefack, C. The and P.H. Amvam Zollo, 1998. La lutte naturalle contre les champignons des semences du maïs à l'aide des huiles essentielles et des extraits de plantes (Natural control of fungi in maize seeds using essential oil and plant extracts). In: Biosciences and Biodiversity, Mbofung, C.M. F., F. X. Etoa, P. Moundipa and J. Ngogang (eds), Proceedings of the Annual Conference of the Cameroon Biosciences Society, December 1998, Buea, Cameroon, 5: 230-234.
15. Tagne, A., J. Nguefack, C. The and P.H. Amvam Zollo, 2000. Natural control of fungi and mycotoxin in grains means of reducing human and animal contamination. Journal of Applied Science of Southern Africa, 6: 37- 45.
16. Raju, C.A. and L. Sangam, 1977. Efficacy of fungicides in controlling seed borne infection of Cephalosporium acremonium and Fusarium moniliforme in maize. Indian Phytopathology, 30: 17-20.
17. Tagne, A., C. The and H.J. Hansen, 1997. Methods of seed treatment and the efficacy of some fungicides in the control of *Fusarium moniliforme* in maize seeds. In Contributing to Food Self Sufficiency: Proceedings of a Regional Maize workshop Cotonou, Benin 29 May-2 June 1995. (eds) B. Badu-Apraku, M.O. Okorada, M. Ouedraogo and F.M. Quin. IITA-WECAMAN, pp: 346-351.
18. ISTA, 1999. International Rules for Seed testing (ISTA) 23: 269.
19. Remmal, A.T., T. Bouchikhi, K. Rhayour, M. Ettayebi and A. Tantaoui-Elaraki, 1999. Improved method for the determination of antimicrobial activity of essentials in agar medium.. Journal of essentials oil Res., 5: 179-184.
20. Adegoke, G.O. and A. Adesola, 1996. Storage of maize and cowpea and inhibition of microbial agents of biodeterioration using the powder and essential oil of lemon grass (Cymbopogon citratus). International Journal of Biodeterioration and Biodegradation, 62: 81-84.
21. Singh, D.V., S.B. Mathur and P. Neergaard 1974. Seed health testing of maize. Evaluation of testing techniques with particular reference to Drechslera maydis. Seed Science and Technology, 2: 349-365.
22. Annonymous, 1998. SigmaStat for Windows 2.0.

23. ISTA, 1979. Handbook for seedling evaluation (ISTA), pp: 130.
24. Naik, D.M., I.N. Nawa and R.H. Raemaekers, 1982. Absence of an effect from internally seed-borne Fusarium moniliforme on emergence, plant growth and yield of maize seed. Seed Science and Technology, 10: 347-356.
25. Wilson, D.O. Jr., S.K. Mohan, E.A. Knott and B. Shafii, 1993. Evaluation of fungicides seed treatments for shrunken-2 (Supersweet) sweet corn. Plant Disease, 77: 348-351.
26. Steven Groot, P.C., M. Jan van der Wolf, Henk Jalink, J. Cees Langerak, W. Rund van den Bulk, 2004. Challenges for the production of high quality organic seeds. Sees testing International, 127: 12-15.
27. Dwivedi, S.K., S.K. Dwivedi, V.N. Pandey, N.K. Dubey, 1991. Effect of essential oil of some higher plants on the Aspergillus flavus Link. Infesting stored seeds of guar (tetragonoloba L.(Taub.). Flavour and Flagrance, pp: 6295-297.
28. Wilson, C.L., J.M. Solar, A. El Ghaouth and M.E. Wisniewski, 1997. Rapid Evaluation of Plant Extracts and Essential oils for antifungal activity against Botrytis cinerea. Pant Disease, pp: 81204-210.
29. Christian, E.J., A.S. Goggi, 2008. Integrating seed treatment in organic and open pollinated corn systems. Report Leopold Center for Sustainable Agriculture, 17: 18-20.
30. Nguefack, J., V. Leth, J.B. Lekagne Dongmo, J. Torp, P.H. Amvam Zollo and S. Nyasse, 2008. Use of three essentials oils as seed treatments against seed-borne fungi of Rice (Oriza sativa L.) Am-Euras. J. Agric and Env., 4(5): 554-560.