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Efficacy of essential oils of *Cymbopogon citratus* (D.C.) Stapf, *Lippia multiflora* Moldenke and hot water in the control of seed-borne fungi *Phoma sorghina* and their effects on *Sorghum bicolor* (L.) Moench seed germination and plants development in Burkina Faso

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ABSTRACT

The effects of two essential oils (*Cymbopogon citratus* and *Lippia multiflora)* and varying durations of hot water at 50°C on seed-borne fungi *Phoma sorghina* on five varieties of sorghum were evaluated using morphological traits. The varieties were ICSV 1001, Sariaso 03, ICSV 1049, Kapelga and the local variety, 1341So07. The results indicate that hot water treatment and essential oil of *C. citratus* significantly reduced the infection level of *P. sorghina* compared to untreated seeds, seeds treated with sterile water and fungicide Calthio C. Essential oil of *L. multiflora*, significantly lowered the infection level of *P. sorghina,* compared to untreated seeds and seeds treated with water in varieties of white grain such as Sariaso 03 and ICSV 1049. Hot water treatment did not have a repressive effect against sorghum seed germination for all the varieties tested. On the contrary, essential oil of *C. citratus* had an inhibitory effect on the germination of seeds of varieties Kapelga and ICSV 1049 compared to untreated seed. On the other varieties tested, it reduced the number of germinated seeds in comparison to untreated seed. According to tested varieties, essential oil of *L. multiflora* had an inhibitory effect (on ICSV 1049) or improved seed germination (on Kapelga). In general, the treatments applied have improved the development of sorghum plants grown from treated seed.

Keywords: Hot water, essential oil, seed treatment, germination, *Phoma sorghina*, sorghum.

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INTRODUCTION

Seed treatment is one of the means of allowing the establishment of healthy crops. This method uses many techniques among which, seed coating by chemical products is the main technique currently applied to control seed-borne fungi (Aubertot et al., 2005). In spite of satisfactory results that this technique gives, studies show that fungicides can give cancer to those who use chemical products without suitable protection. In addition, fungicides are not suitable to control fungi in grain for human consumption and in fruits because of their toxicity to humans. Also, grain and fruit are also infected by fungi that can secrete, during period of infection, mycotoxin dangerous for human and animals (Galvano et al., 2001). A significant portion of the agricultural produce in the country and the world over becomes unfit for human consumption due to mycotoxin contamination of grain (Majumder et al., 1997). Fungicides also have negative consequences on the environment. They can pollute

water and kill many useful micro-organisms in the soil (Pimentel and Levitan, 1986). Regarding harmful effects of fungicides on human and environment, the search of better methods that are inexpensive and safe for man and the environment must be considered.

Hot water seed treatment is one of the natural methods that allow eliminating seed-borne bacteria and fungi. This technique has been successful in the treatment of avocado against *Phytophtora cinnamomi* (Zentmyer et al., 1958). Hot water is also recommended for own seeds treatment to prevent seed-borne diseases such as black rot, black leg, black spot and ring spot of crucifers. This has reduced the infection level of pathogens such as *Alternaria* spp., *Colletotrichum* spp., *Phoma* spp. and bacteria pathogen like *Pseudomonas* spp. and *Xanthomonas* spp. (Nega et al., 2003). The heat treatment of 10 min in water at 55°C of potato tubers, completely controlled blackleg infection in the field (Mackay and Shipton, 1983). Research has shown that hot water treatment can penetrate the seed sufficiently to eradicate pathogens inside the seed (Babadoost, 1992; Boucher et al., 1995). Beyond hot water treatment, the effectiveness of plant extracts such as essential oil is well documented (Isman, 2000; Zaridah et al., 2003; Kordali et al., 2005; Dhaliwal and Koul, 2007; Somda et al., 2007; Koul et al., 2008). These products are biodegradable and mostly non-toxic to mammals, birds and fish (Stroh et al., 1998).

Hot water treatment and plant-based pesticides can therefore be alternatives due to their minimal effect on the environment and non-toxicity to consumers. On this basis, we report our findings on the efficacy of hot water and essential oil on seed treatment and transmission of *P. sorghina*, a seed-borne fungus of sorghum, a very important cereal in Burkina Faso.

MATERIALS AND METHODS

Sorghum samples

Five varieties of sorghum were used and include ICSV 1001 with red pericarp, Sariaso 03, ICSV 1049 and Kapelga white pericarp obtained from sorghum improvement program, INERA Farakobâ and the local variety, 1341 So07 which has red pericarp from Diébougou in Burkina Faso.

Plant materials, seed treatment and experimental design

C. citratus (D.C.) Stapf is an aromatic inveterate grass of the family Poaceae cultivated for its medicinal properties and essential oils used in cosmetics and perfumery. On the other hand, *Lippia multiflora* Moldenke commonly called savanna tea (Arbonnier, 2002) belongs to the family *Verbenaceae.* The leaves are used to treat malaria and coughs (Nébié, 2006).

The essential oils of *C. citratus* and *L. multiflora* used were supplied by Dr. Nébié Roger Charles Honorat a chemist of IRSAT/Ouagadougou, Burkina Faso. The essential oil was first emulsified using sterile solution of agar at 0.1% and diluted with sterile water (8%) before use. The solution of the oils were applied at 100 µl for 1 g of seeds, thoroughly mixed in pot, tightly covered and kept in the laboratory between 28 and 30°C for 24 h.

The experimental design was a completely randomized block design with 8 treatments as follows: untreated seeds, seed treated with hot water, fungicide, essential oils of *C. citratus* and *L. multiflora* and hot water at 50°C for 30, 40 and 50 min. At the end of the time of hot water treatment, the tubes were removed and the seeds were aseptically dried on sterile filter papers with high water-holding capacity under a Lamin air flow bench. Each treatment was repeated 8 times.

Plating and incubation of seeds

Two hundred sorghum seeds were used for each treatment and 25 seeds were plated in each Petri dish containing three wet filter papers. After plating, the Petri dishes were incubated under 12 h alternating cycles of near ultraviolet (NUV) and darkness at 22°C for seven days.

Planting of seeds in pots

The seedling substrate used was white sand from the sandpit of 'Kôrô'. The sand was sterilized and allowed to cool, then put in pots. Each pot had 25 seed holes of 1 cm deep and 0.9 cm in diameter arranged in circles, 15 seed holes in the outer ring, 9 in the middle ring and one in the center. One seed was planted in the holes. The pots were watered 24 h after planting and subsequently as needed. A total of 200 seeds were used by treatment.

Evaluation and analysis

After incubation, the seed were examined under a compound microscope to identify fungi developing on the different seeds and results were recorded on the seed health report.

The effect of treatment on the germination of sorghum seeds is evaluated by counting the number of seeds germinated 10 days after sowing. The vigor of the plant was evaluated 14 days after sowing by weighing (cg) and measuring the height (cm) of 5 well developed plants taken from each treatment.

Data were recorded in Microsoft Excel and analyzed using one-way analysis of variance (ANOVA) with the SPSS 16.0 Software. Significant differences between treatments, means separation were carried out with the multiple classification of Duncan at 5% probability.

RESULTS

Efficacy of treatments on seed-borne fungi of sorghum

Hot water treatment at 50°C for 30, 40 and 50 min, fungicide Calthio C and essential oils of *C. citratus* significantly reduced the infection rate of *P. sorghina* compared to untreated seeds and seeds soaked in sterile water and essential oil of *L. multiflora* in all the samples Except essential oil of *L. multiflora* on ICSV 1001 and 1341So07, essential oil of *C. citratus* and hot water treatment were not significantly different from fungicide Calthio C in the reduction of *P. sorghina* in all the tested samples. Therefore, essential oil of *L. multiflora* was not effective in controlling *P. sorghina* on varieties of red seed. On the other hand, efficacy of *C. citratus* did

Table 1. Efficacy of essential oil and hot water treatment against *P. sorghina* a sorghum seed borne fungus.

Treatments	Infection rate of *P. sorghina* in sorghum seed				
	ICSV1001	ICSV1049	Sariaso 03	Kepelga	1341So07
Water treatment	34^{b}	62^{b}	73^{c}	28^{b}	62^{c}
Untreated seeds	37^{b}	49^{b}	54^{b}	37^{b}	48^{c}
Fungicide	1^{a}	2^{a}	3^{a}	2^{a}	0^{a}
50°C for 30 min	1^{a}	1^{a}	4^{a}	0^{a}	2^{a}
50°C for 40 min	0^{a}	0^{a}	2^{a}	0^{a}	0^{a}
50°C for 50 min	1^{a}	0^{a}	1^{a}	0^{a}	0^{a}
EO *C. citratus*	0^{a}	3^{a}	3^{a}	0^{a}	0^{a}
EO *L. multiflora*	42^{b}	3^{a}	3^{a}	2^{a}	16^{b}

50°C for 30 min: Seeds soaked in sterile water at 50°C for 30 min, 50°C for 40 min: Seeds soaked in sterile water at 50°C for 40 min. 50°C for 50 min: Seeds soaked in sterile water at 50°C for 50 min EO: Essential oil. The means followed by the same letter in the same colon are not significantly different at the level at 5% according to multiple classifications of Student-Newman-keuls.

Table 2. Effects of hot water and essential oil on sorghum seed germination.

Treatments	Percentage of seed germination				
	ICSV1001	ICSV1049	Sariaso 03	Kepelga	1341 So07
Water treatment	78	90^{b}	77^{abc}	82^{bc}	59^{ab}
Untreated seeds	62	86^{b}	74^{ab}	72^{b}	52^{a}
Fungicide	71	90^{b}	89^{c}	94^{c}	65^{bc}
50°C for 30 min	70	91^{b}	85^{bc}	92^{c}	76^{c}
50°C for 40 min	67	88^{b}	81^{bc}	87^{c}	71^{bc}
50°C for 50 min	70	81^{ab}	89^{c}	92^{c}	72^{c}
EO *C. citratus*	57	73^{a}	65^{a}	49^{a}	50^{a}
EO *L. multiflora*	66	73^{a}	76^{bc}	85^{c}	52^{a}

50°C for 30 min: Seeds soaked in sterile water at 50°C for 30 min; 50°C for 40 min: Seeds soaked in sterile water at 50°C for 40 min; 50°C for 50 min: Seeds soaked in sterile water at 50°C for 50 min; EO: Essential oil. The means followed by the same letter in the same colon are not significantly different at the level at 5% according to multiple classifications of Student-Newman-keuls.

not vary according to the color of the sorghum seeds. Hot water treatment and essential oil of *C. citratus* and *L. multiflora* also significantly reduced the infection rate of other seed-borne fungi like *Fusarium* spp., *Curvularia* spp., *Colletotrichum graminicola*, *Bipolaris* spp. (Table 1).

Effect of treatments on sorghum seed germination and plant vigour

The essential oils tested lowered the number of germinated seeds in all the tested samples. The essential oil of *C. citratus* significantly reduced the number of germinated seeds in ICSV 1049 and Kapelga in comparison with untreated seeds and seeds treated with sterile water. The essential oil of *L. multiflora* significantly lowered seed germination in varieties ICSV 1001 compared to untreated seeds. Hot water treatments improved seeds germination compared to untreated seeds and seeds treated with sterile water. It significantly increased the number of germination in 1341So07 which is naturally infected at 68% by *P. sorghina* compared to untreated seeds and seeds treated with sterile water. In the samples of Sariaso 03 and 1341So07 hot water treatment significantly increased the number of germinated seeds compared to essential oil of *C. citratus.* Similar results were obtained between hot water treatments and essential oils of *C. citratus* and *L. multiflor* with variety ICSV (Table 2).

Effect of hot water treatments and essential oils on the weight of plants

The results obtained for the weight of the 5 well developed plants of variety ICSV 1049 indicate that hot water treatment and essential oils of *C. citratus* and *L. multiflora* improved the development of sorghum plants more than untreated seeds and seeds treated with water. For 1341So07 except hot water treatment at 50°C for 40 min, other treatments significantly improved plant development more than fungicide Calthio C. Hot water

Table 3. Effects of hot water and essential oil on sorghum plants weight.

Treatments	Weight (cg) of five plants of sorghum				
	ICSV1001	ICSV1049	Sariaso 03	Kepelga	1341So07
Water treatment	96	34[a]	63	37	32[a]
Untreated seeds	79	40[a]	64	39	34[a]
Fungicide	88	48[bc]	60	33	41[b]
50°C for 30 min	89	44[ab]	66	37	52[d]
50°C for 40 min	104	56[c]	65	38	44[bc]
50°C for 50 min	91	48[bc]	79	36	49[cd]
EO *C. citratus*	120	57[c]	0.57	41	46[c]
EO *L. multiflora*	0.8	0.48[bc]	0.63	0.38	0.5[cd]

50°C for 30 min: Seeds soaked in sterile water at 50°C for 30 min; 50°C for 40 min: Seeds soaked in sterile water at 50°C for 40 min; 50°C for 50 min: Seeds soaked in sterile water at 50°C for 50 min; EO: Essential oil. The means followed by the same letter in the same colon are not significantly different at the level at 5% according to multiple classifications of Student-Newman-keuls.

Table 4. Effects of hot water and essential oil on sorghum plants height.

Treatments	Sorghum plant height (cm)				
	ICSV1001	ICSV1049	Sariaso 03	Kepelga	1341So07
Water treatment	19.98[bc]	12.88[a]	19	14.5[b]	14.12[a]
Untreated seeds	18.39[ab]	12.5[a]	17.75	13.62[ab]	13.06[a]
Fungicide	16.68[a]	14.12[ab]	18.8	13.25[a]	13.38[a]
50°C for 30 min	18.70[ab]	15[bc]	19	14.75[abc]	16[b]
50°C for 40 min	20.37[bc]	16.88[d]	18.88	14.88[c]	15.75[b]
50°C for 50 min	21.5[c]	16.5[cd]	19.62	13.25[a]	15.5[b]
EO *C. citratus*	20.5[bc]	16.65[cd]	17.88	15.3[c]	15.75[b]
EO *L. multiflora*	18.5[ab]	21.52[bcd]	19	15.25[c]	16.25[b]

50°C for 30 min: Seeds soaked in sterile water at 50°C for 30 min; 50°C for 40 min: Seeds soaked in sterile water at 50°C for 40 min; 50°C for 50 min: Seeds soaked in sterile water at 50°C for 50 min; EO: Essential oil. The means followed by the same letter in the same colon are not significantly different at the level at 5% according to multiple classifications of Student-Newman-keuls.

treatment and essential oils significantly increased plants weight compared to plants raised from untreated seeds and seeds treated with sterile water. There was no significant difference among treatments in varieties ICSV 1001 and Sariaso 03 (Table 3).

Effects of treatments on the height of sorghum plants

Generally, hot water treatment and essential oils favoured the height of plants obtained from treated seeds more than plants grown from seeds treated with fungicide and untreated seeds. In 1341So07 and ICSV 1049 varieties, hot water treatments and essential oils significantly improved plants height more than plants obtained from untreated seeds, seeds treated with sterile water and fungicide calthio C except essential oil of *L. multiflora*. Hot water treatment at 50°C for 50 min resulted in increase of plant height of variety (ICSV 1001) as compared to fungicide Calthio C and plants obtained from untreated seed. For the same variety (ICSV 1001), hot water treatment at 50°C for 40 min and essential oil of *C. citratus* significantly improved plants height compared to fungicide Calthio C. In Kapelga variety, essential oils and hot water treatment at 50°C for 40 min improved the height of plants more than fungicide and untreated seeds (Table 4).

DISCUSSION

Hot water treatments of sorghum seed at 50°C for 30, 40 and 50 min, cleared seed of 5 varieties of sorghum from *P. sorghina* infection. In addition, hot water treatments improved seed germination and plant development. Previous reports indicated that hot water treatments eliminate fungal species [*Fusarium* spp., *Curvularia* spp., *Alternaria* spp., *Colletotrichum graminicola* (Ces.) Wilson, *Bipolaris* spp. ref] in naturally infected seeds of sorghum. Hot water treatments also improved sorghum seeds germination and development of plants obtained from treated seed. Zentmyer et al. (1958) showed that hot

water treatment at 120°F for 30 min controlled root rot fungus, *Phytophthora cinnamomi* Rands, from avocado seed. In the greenhouse and in the field, trials indicated no retardation in the rate of germination or reduction in the percentage of germination of treated seed. On crucifers, hot water treatment is effective in eliminating seed-borne fungi such as *Alternaria* spp., *Phoma* spp., *Septoria* spp. and pathogenic bacteria such as *Pseudomonas* spp. and *Xanthomonas* spp. Nega et al. (2003) and Mackay and Shipton (1983), indicated that hot water treatment of potato tubers naturally infected by *Erwinia carotovora* subsp. *atroseptica* resulted in the complete absence of blackleg infection in the field. The use of hot water in seed requires that the specific temperature and time intervals be followed strictly, in order to maintain seed viability.

Essential oil of *C. citratus* at 8% was effective in the control of *P. sorghina* in naturally infected sorghum seed. At this proportion, essential oil of *C. citratus* lowered sorghum seed germination. Somda et al. (2007) showed that essential oil of *C. citratus* at 6% was effective against *C. graminicola* and *P. sorghina* and did reduce germination of treated seed. These results show that essential oil of *C. citratus* contains toxic substances and the toxicity of this oil increases with the proportion of oil used. Essential oil of *L. multiflora* also completely eliminated *P. sorghina* in sorghum samples of white seeds, but not in sorghum samples of red seed. The presence of anthocyanic and tannin substances can explain the decrease in antifungal substance absorption. Essential oil of *L. multiflora* improved sorghum seeds germination in most of the varieties tested. The results of weight and height of sorghum plants obtained from seed treated with essential oil revealed that they improve plant development because treatment eliminated most of the pathogenic fungi offering to future plants good conditions for their development.

Conclusion

Seed treatment with essential oil and hot water significantly reduce sorghum seed infection by fungi. Seed treatment with hot water also improves sorghum seed germination, but those of essential oils slightly reduce sorghum seeds germination in comparison with untreated seeds and seeds treated with sterile water. Sorghum plants obtained from seeds treated with hot water and essential oils grow very well than those coming from untreated seeds and seeds treated with sterile water.

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REFERENCES

Arbonnier M, 2002. Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. 2e éd. CIRAD et MNHM, pp. 173.

Aubertot JN, Barbier JM, Carpentier A, Gril JJ, Guichard L, Lucas P, Savary S, Savini I, Voltz M, 2005. Pesticides, agriculture et environnement. Réduire l'utilisation des pesticides et limiter leurs impacts environnementaux. Expertise scientifique collective, synthèse du rapport, INRA et Cemagref (France), 64 p.

Babadoost M, 1992. Vegetable seed treatment. RPD N° 915.

Boucher J, Hazzard R, Wick R, 1995. Integrate Pest Management: Bacteria Leaf Spot of Peppers. University of Connecticut Coopperartive Extension System, England (Accessed on July 25, 2013).

Dhaliwal GS, Koul O, 2007. Biopesticides and Pest Management: Conventional and Biotechnological Approaches. Kalyani Publishers, New Delhi.

Galvano F, Piva A, Ritieni A, Galvano G, 2001. Dietary strategies to counteract the effect of mycotoxins: a review. J Food Prot, 64: 120-131.

Isman MB, 2000. Plant essential oils for pest and disease management. Crop Prot, 19:603-608.

Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A, 2005. Screening of chemical composition and antifungal activity of essential oils from three Turkish *Artemisia* species. J Agric Food Chem, 53:1408-1416.

Koul O, Walia S, Dhaliwal GS, 2008. Essential oils as green pesticides: potential and constraints. Biopestic Int, 4(1):63-84.

Mackay JM, Shipton PJ, 1983. Heat treatment of seed tubers for control of potato blackleg (Erwinia carotovora subsp. Atroseptica) and other diseases. Plant Pathol, 32:385-393.

Majumder UK, Gupta M, Mukhopadhyay DK, 1997. Effect of mycotoxins isolated from *Penicillium nigricans* on glucose-6-phosphate dehydrogenase. Indian J Exp Biol, 35:1233–1236.

Nébié RCH, 2006. Etudes des huiles essentielles de plantes aromatiques du Burkina Faso : Production, Composition chimique et Propriétés insecticides. Thèse de Doctorat d'Etat ès Sciences Physiques (Chimie Organique : structure et réactivité), Université de Ouagadougou, Burkina Faso. Pp. 175.

Nega E, Ulrich R, Werner S, Jahn M, 2003. Hot water treatment of vegetable seed an alternative seed treatment method to control seed borne pathogens in organic farming. J Plant Dis Prot, 110(3):220-234.

Pimentel D, Levitan L, 1986. Pesticides: Amounts applied and amounts reaching pests. Bioscience, 36:86-91.

Somda I, Leth V, Sereme P, 2007. Antifungal effect of *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Azadirachta indica* oil extracts on sorghum seed-borne fungi. Asian J Plant Sci, 6:1182-1189.

Stroh J, Wan MT, Isman, MB, Moul DJ, 1998. Evaluation of the acute toxicity to juvenile Pacific coho salmon and rainbow trout of some plant essential oils, a formulated product, and the carrier. Bull Environ Contam Toxicol, 60:923-930.

Zaridah MZ, Nor Azah MA, Abu Said A, Mohd Faridz ZP, 2003. Larvicidal properties of citronellal and *Cymbopogon nardus* essential oils from two different localities. Trop Biomed, 20:169-174.

Zentmyer GA, Thorn WA, Paulus AO, Burns RM, 1958. Hot-water treatment of Avocado seed. California Avocado Soc, 42:108-110.