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Effect of Seed Treatment with Some Essential Oils on Fungal Incidence and Seed Germination of Wheat

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Abstract: Fungi are among the major factors causing post-harvest deterioration of crop produce worldwide. Overall storage fungi have remained an important constraint to wheat production all over the world including in Ethiopia. However, effective and sustainable management of the storage fungi is needed to be achieved under Ethiopian condition. Therefore, the current work was carried out to evaluate the effect of seed treatment with some essential oils on fungal incidence and seed germination of wheat. For this purpose, five essential oils namely Rosmary (*Rosmarinus officinalis*), Lemongrass (*Cympobogon citratus*), Lavender (*Lavandula anguistifolia*), Palmarosa (*Cympobogon martini*) and Citronella (*Cympobogon nardus*) were tested for their inhibition against fungal incidence and seed germination of wheat. All the tested oils caused inhibition of the fungal incidence and seed germination albeit at different rates. Lemongrass, Lavender, Palmarosa and Citronella resulted in complete fungal contamination and seed germination inhibition of wheat at the lowest minimum concentrations of 25 µL per 10 mL of PDA media. Similar effects were observed for Rosemary oil, when the concentration increases to the highest concentration of $1.25X10^{-3}$ (V/V). The tested oils could play an important role in reducing fungal contamination and have the potential to be used as an alternative or component in integrated storage fungi management. However, the detrimental effects of essential oils were observed on wheat seed germination. It is suggested that large scale screening of oils on fungal incidence and seed germination capacity should be investigated and confirmed with additional studies.

Key words: Essential Oils · Fungal Incidence · Seed Germination, Storage Fungi · Wheat

INTRODUCTION

The grain production varies from year to year and hence the grains should be stored strategically from years of overproduction for the use in year of under production. Also grain must be stored as point of production is not the point of consumption and the time of production is not the time of consumption. Stored grains can have losses in both quantity and quality. Grain quality after harvest is influenced by a wide variety of biotic and abiotic factors and has been studied as a stored grain ecosystem. Losses occur when the grain is attacked by microorganisms and other organisms including insects, mites, rodents and birds. The grain losses in quantity and quality; can be in the form of depletion in seed viability, hardness, color, size and shape, grain weight and various biochemical parameters viz., protein, carbohydrate and vitamins under postharvest storages. The storage fungi damage the grains in several ways; they reduce the germination capacity, produce undesirable odor and kernel discoloration, decrease the food value and also produce toxins that are injurious to the health of consumers [1].

Overall storage fungi have remained an important constraint to wheat production all over the world including in Ethiopia. However, effective and sustainable management of the storage fungi is needed to be achieved under Ethiopian condition. Fungal growth and mycotoxin

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production in cereals is influenced by various factors. Climatic conditions, especially temperature and humidity, play a very important role in this process [2]. Microscopic fungi and their metabolites, mycotoxins, are often found as contaminants in agricultural products before or after harvest as well as during transportation or storage. Furthermore, infection rate of seeds depends on some environmental conditions such as high relative humidity, suitable temperature and also high level of moisture content in seed. It is difficult to get the exact information on post-harvest losses of grains in Ethiopia but the frequently used figure ranges from 20-30% [3].

The development of relevant management strategies are lacking. Chemical fungicides commonly used to control growth of fungi in storage may not be appropriate in many occasions, because of their several side effects. Natural alternatives that are user friendly and demonstrate low toxicity to humans and animals should be identified. In Ethiopia, in spite of the presence of wide diversity of plants and their essential oils with antifungal properties, no or little researches have been done [4]. Despite its importance, storage fungi has been one of the most poorly understood patho-systems in Ethiopia. So far, only limited surveys were conducted in few areas. Yield loss assessment studies have been carried out in fewer areas and they are largely based on data from field surveys. Lack of post-harvest technologies causes on an average 15-20% losses due to pests and climatic factors [4]. As a result there is a need to assess the incidence and frequency of storage fungi in different areas and across agro-ecological zones. Furthermore, various storage fungi management practices should be tested and recommended. Thus, this study was designed to evaluate the effect of seed treatment with some essential oils on fungal incidence and seed germination of wheat.

MATERIALS AND METHODS

Plant Materials and Extraction Methods: Essential oils were extracted from five plant materials (Table 1). These plant materials from Wondo Genet agricultural research center were reported to having antimicrobial activities [5].

The essential oil from the five plant species was extracted by hydro distillation at the Essential Oil Research Center (EORC/Wondo Genet) of the Ethiopian Institute of Agricultural Research (EIAR). Samples were subjected to moisture content determination using oven-dry method at 105ºC. Two hundred gram dried herbs from each sample were ground and pulverized to be admixed with 100-200 mL water placed in a distillation flask at room temperature. The distillation process took 3 hours. The distillate was collected in a separating funnel of Clavenger apparatus in which the aqueous portion was separated from volatile oil [6].

Effect of Plant Extracts on Fungal Contaminations and Seed Germination: Untreated and plant extract-treated grains of wheat were used for laboratory experiments. Grains were soaked in each extract at concentrations of 25µl, 50µl, 75µl, 100µl and 125µl for one hour, blotted dry and ten grains were plated on PDA. The extract treated and untreated grains were incubated at 25°C for seven days. Grains plated on PDA were examined for percent fungal contamination and percentage grain germination, after four days for every 48 hours interval and after eight days of incubation. Treatments were arranged in completely randomized design with five replicates of 10 seeds [7].

Percentage Inhibition: The minimum inhibitory concentration of oils was calculated with respect to the control according to the following formula described by Baratta [8] as follows:

$$Inhibition\ (\%) = \frac{(C - T)}{C} X100$$

where,

C = Mean fungal growth in control treatments,

T = Mean fungal growth in tested oils.

Germination Percentage (G %): The proportion of seeds that develop normal seedlings. The relative reduction of germination was recorded by comparing the relative amount of germination capacity.

$$G\ (\%) = \frac{Numbers\ of\ grain\ germinated}{Total\ number\ of\ grain} X100$$

Statistical Analysis: One way analysis of variance was carried out by using the general linear model procedure and test was performed at a probability level of (P = 0.05). Comparison between means of data for fungal incidence and grains germination inhibition was done to determine significance differences among different treatments of plant extracts and concentrations. Treatment means were separated by using least significant difference (LSD) test at 5% significance level using SAS software package [9].

Table 1: Plant Materials Used for the Extraction of Essential Oils for Antifungal Tests

Scientific name	Common name	Plant type	Bioactivity	References
Rosmarinus officinalis	Rosmary	Herb	Antimicrobial, medicinal	[5]
Cympobogon martini	Palmarosa	Herb	Antimicrobial, antimalarial	[5]
Lavandula anguistifolia	Lavender	Herb	Antimicrobial	[5]
Cympobogon citratus	Lemongrass	Herb	Antimicrobial, antioxidant	[5]
Cympobogon nardus	Citronella	Herb	Antimicrobial, medicinal	[5]

RESULTS AND DISCUSSIONS

The Effect of Seed Treatment with Essential Oils on Fungal Incidence: Essential oils tested in the current experiment significantly reduced fungal incidence (*P=0.05*) compared to the control (Table 2). Fungal incidence of grains treated with Lemongrass, Palmarosa and Citronella was nil, whereas reduced fungal incidences were observed for Lavender followed by Rosemary. The effect of Rosemary at concentrations of 0.75% and 1% were not significantly different when compared to lavender at concentration of 0.25%. In addition, at concentration of 1.25% no significant difference between Rosemary and Lavender at 0.5%, 0.75%, 1% and 1.25%.

The effect of Rosemary was not significantly different between 0.25% and 0.5% and also performed equally both at 0.75% and 1%. At concentration of 1.25% fungal incidence inhibition was significantly different and higher than the lower concentrations. On the other hand, the effect of lavender was not significantly different between 0.5%, 0.75%, 1% and 1.25% and also performed equally at 0.75%, 1% and 1.25%. At concentration of 0.25% fungal incidence inhibition was significantly different and lower than the higher concentrations. These indicated that these oils exhibited strong fungal incidence inhibition and as the concentration increase fungal incidence decrease (Table 2).

The Effect of Seed Treatment with Essential Oils on Seed Germination: Germination of wheat seeds after treatment with essential oils varied significantly (*P=0.05*) across treatments; therefore, the essential oils did have phytotoxic effects (Table 2). Seed germination of grains treated with Lemongrass, Palmarosa and Citronella was nil, whereas reduced seed germinations were observed for Lavender followed by Rosemary. Also at concentration of 0.25% and 0.5% significant differences were observed between Rosemary and other oils. However, no significant difference of Rosemary was observed at concentration of 0.75% and 1% compared to Lavender at concentration of 0.25%, 0.5%, 0.75% and 1%. On the other hand, the effect of Rosemary and Lavender at concentrations of 0.25%, 0.5% and 1.25% were significantly different.

Table 2: The Effect of Seed Treatment with Essential Oils on Fungal Incidence and Seed Germination

Treatment		Inhibition (%)	
Essential Oil	Concentration (µl)	Incidence (%)	Germination (%)
	0	100.00^{A}	100.00^{A}
	25	63.33^{B}	50.00^{B}
RM	50	60.00^{B}	43.33^{B}
	75	43.33^{C}	23.33^{C}
	100	43.33^{C}	23.33^{C}
	125	26.67^{D}	23.33^{C}
	0	100.00^{A}	100.00^{A}
	25	0.00^{F}	0.00^{D}
LG	50	0.00^{F}	0.00^{D}
	75	0.00^{F}	0.00^{D}
	100	0.00^{F}	0.00^{D}
	125	0.00^{F}	0.00^{D}
	0	100.00^{A}	100.00^{A}
	25	40.00^{C}	23.33^{C}
LV	50	20.00^{ED}	16.67^{C}
	75	16.67^{E}	16.67^{C}
	100	16.67^{E}	16.67^{C}
	125	16.67^{E}	0.00D
	0	100.00^{A}	100.00^{A}
	25	0.00^{F}	0.00^{D}
PR	50	0.00^{F}	0.00^{D}
	75	0.00^{F}	0.00^{D}
	100	0.00^{F}	0.00^{D}
	125	0.00^{F}	0.00^{D}
	0	100.00^{A}	100.00^{A}
	25	0.00^{F}	0.00^{D}
CT	50	0.00^{F}	0.00^{D}
	75	0.00^{F}	0.00^{D}
	100	0.00^{F}	0.00^{D}
	125	0.00^{F}	0.00^{D}
Mean		28.22	24.55
CV		16.28	28.23
LSD (5%)		7.5	11.32

RM=Rosemary, LG= Lemongrass, LV= Lavender, PR= Palmarosa, CT= Citronella

*0= No fungal growth were observed

*Means with the same letters are not significantly different (p = 0.05)

The effect of Rosemary was not significantly different at concentrations between 0.25% and 0.5% and between 0.75%, 1% and 1.25%. On the other hand, the effect of lavender was not significantly different at all concentrations except at 1.25%. This indicates that these oils reduced or totally inhibited germination of the seeds (Table 2).

Many reports have shown the importance of plant extracts for controlling seed-borne fungi of wheat grains [10-12]. Thyme oil showed the highest inhibition, followed by rosemary and laurel of *A. parasiticus* and *A. flavus* mold growth on wheat and maize grains [13]. The inhibitory effect of the tested extracts might be due to natural bioactive materials present in these extracts [14]. The experiments indicated that lemongrass essential oil could be employed as alternate to synthetic fungicides during grain storage [15]. For one hour before sowing or storage, the aqueous extract of *A. sinaica* can be used to treat wheat grains, to reduce the fungal incidence. Aqueous extracts of the aerial parts of selected medicinal plants, particularly *A. sinaica*, are promising for protecting Egyptian wheat grain cultivars against major seed-borne fungi and the aqueous extracts are expected to improve crops [16].

The allelopathic effects of these oils were reported in previous studies, presenting a risk or advantage to seed germination and seedling growth suggesting that proper doses of these essential oils could be used. Both essential oils of Oregano and Rosemary caused a generally detrimental effect on seed germination rate, seedling shoot length, seedling root length and seedling fresh weight of wheat cultivars. Oregano and Rosemary oils caused 37 to 87% and 10 to 78% germination inhibition on wheat cultivars, respectively [17, 18]. However, this finding is not agreed with Fatu [19], which states that the effect of these oils without the detrimental effect on seed germination should be further investigated and need to be confirmed with additional studies.

Monoterpenes present in essential oils are very powerful germination inhibitors to wheat seeds. One of the major compound of rosemary essential oil is 1, 8-cineole. Asanova [20] demonstrated that this component has very strong cytotoxicity. It is possible that this component has the major role in cytotoxicity of this essential oil. 1, 8 - cineole exhibits a strong toxic effect on eukaryotic cells [21, 22]. Limonene, one of the components of rosemary essential oil, has ability to moderate proliferation of cells [23] and in that manner it affects cell mitosis.

Evidence for allelopathic interactions in nature caused by aromatic plants containing volatile allelochemicals have been described frequently [24, 25]. Essential oils were reported as inhibitors of seed germination and plant growth [26]. It was previously reported on the effect of monoterpenes from a number of aromatic plants on the germination of wheat [27, 28] and have also shown that wheat seeds exposed to defined monoterpenes, such as citral, citronellal, pulegone and carvacrol are able to metabolize it [29]. According to all this facts, it is not surprising that low concentration of essential oils (EOs) totally inhibited germination of wheat grains.

CONCLUSION AND RECOMMENDATION

Storage fungi has been one of the most poorly understood pathosystems in Ethiopia. As a result, the present study was conducted to determine the incidence of storage fungi in wheat to have a complete picture on the importance of the storage fungi in the country. The major objective of the study was to contribute towards improved wheat production in Ethiopia through effective and sustainable management of storage fungi in the country.

In vivo tests on the efficacy of essential oils against fungal grain contamination proved that all the tested oils had inhibitory effects in the current experiment. Generally, Lemongrass, Lavender, Palmarosa and Citronella oils were the most effective against the fungi as they caused complete fungal mycelia radial growth inhibition at the lowest minimum concentration (0.25% V/V). The same effects were observed for Rosemary oil when the concentrations increased to 1.25% V/V. The tested essential oils also resulted in significant reduction of fungal incidence after seed treatment. However, among oils Lemongrass, Palmarosa and Citronella also affected seed germination at the lowest minimum concentration of (0.25%). The same effects were observed among Rosemary and Lavender oils when the concentrations increase to 1.25% suggesting the need to test lower concentrations that can effectively inhibit fungal infection while not affecting seed germination.

Generally, the tested oils have the potential to be used as an alternative or component in integrated strategies of storage fungi management. However, large scale screening on mycelia radial growth, fungal incidence, mycotoxin production and seed germination capacity should be investigated and need to be confirmed with additional studies.

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