EFFECT OF SEED TREATMENT WITH ORGANIC ACIDS ON THE CONTROL OF COMMON BUNT (*TILLETIA TRITICI* AND *T. LAEVIS*) IN WHEAT

B. SAIDI[1], F. AZMEH[1], O.F. MAMLUK[1] & R.A. SIKORA[2]
[1] Department of Plant Protection, Damascus University, Syria
[2] Institut für Pflanzenkrankheiten, Bonn University
Nussallee 9, D-53115 Bonn, Germany.

ABSTRACT

Common bunt caused by *Tilletia tritici* and *T. laevis* is an important disease, causes considerable losses in wheat yield on a world wild. The disease reduces yields, complicates harvesting and lowers the quality of the grain. It occurs more frequently and causes greater damage on winter wheat than on spring wheat. Grain standards designate wheat that has an unmistakable odor of smut or that contains smut balls, portions of balls, or spores of smut in excess of a quantity equal to 14 balls of average size in 250 g of wheat as "light smutty". Samples containing an excess of 30 balls, or their equivalent, in 250 g of wheat are graded as "smutty". Grain in these grades brings lower prices. Using commercial acetic acid and lactic acid, the pathogen was successfully controlled, but the treatment negatively affected seed germination and seedling vitality. Using dilutions of acetic acid and lactic acid, significant control of the pathogen also was achieved with acetic acid without causing phytotoxicity. Dilutions of lactic acid also gave good control, but showed some phytotoxicity. Using 30-50 ml/kg of vinegar, which is a natural source of acetic acid, proved to be one of the most effective alternatives for control of common bunt on wheat. The treatment had no negative effects on seed germination nor on seedling vitality.

INTRODUCTION

Tilletia tritici (*T. caries*) and *Tilletia laevis* (*T. foetida*) are the causal organisms of common bunt of winter wheat (*Triticum aestivum* L.) as Where *T. tritici* is distributed world-wide wherever wheat is grown, *T. laevis* has a somewhat narrower distribution. It is prevalent throughout Europe and central and eastern north America. The heads affected by common bunt have a distinct blue cast. At bloom, infected heads are more slender than healthy heads and do not put out pollen sacs. At maturity they appear plumper but lighter in weight than normal heads. The smut ball consists of a mass of foul-smelling, dark-brown spores of the stinking smut fungus. In the field, smutted heads usually stand more nearly erect than healthy heads, because of their lighter weight. In some varieties, it is necessary to crush the kernels to determine if heads are diseased. The offensive odor indicates the presence of heavy infections either in the field or in shipped grain. Infected wheat is usually rejected before shipment. While smut balls may be removed by cleaning seed, spores will still be carried on kernels (Kaufman, 1996). When contaminated seeds are sown, the spores germinate in synchrony with the kernels and infect the germinating plants systemically and thereby complete their life cycle.

Ever since seed treatment with organic mercury started in the 1920s, research on this disease has been limited, since this treatment is both cheap and effective. Mercury is now banned in most industerized countries for environmental reasons and other synthetic pesticides have taken its place in the control of bunt. Seed treatments with fungicides remain the most effective deterrent of the common bunt fungi (Hoffmann, 1982; Line, 1993). However, the development of alternate disease management strategies is important, partly because of the perceived health and environmental risks associated with chemical pesticides (Jacobsen and Backman, 1993). Furthermore, there is a possibility of selecting strains of the bunt fungi resistance to chemicals as occurred with hexachlorobenzene (Kollmorgen and Jones, 1975).

Research in ecological regulation of this disease are going on in Europe, mainly focusing on different seed treatments like plant extracts, organic compounds, hot water treatment and antagonistic bacteria (Heyden *et al.*, 1997; Spiess and Dutschke, 1992; Becker and Weltzien, 1993; Bergman, 1996; Bogen and Kristensen, 1996; Gerhardson *et al.*, 1996).

In the present study investigations that were based on natural organic substances easily avialable to the grower.

MATERIALS AND METHODS

Seeds of durum wheat (Cham 4) a winter wheat variety known to be very susceptible to common bunt were contaminated with teliospores of *Tilletia tritici* and *T. laevis* (1:1 w/w) at a rate of 2 g spores/kg seed. Spores were obtained after gentle crushing of the spore balls and sieving. Spores were sprayed around the interior layer of plastic bag, then the seeds were added and mixed together with spores in a rotary movement for the mixture till ensuring a homogenous distribution of spores on seeds.

IN VIVO EXPERIMENTS

For the season (1998-1999), 100 g of contaminated seed were treated with different organic compounds. A commercial product of acetic acid and lactic acid (Merck production) were tested in three levels 0.25, 0.5 and 1 ml/100 g seed. Seeds were sprayed with acids. The treated dried seed then sown two 2 m/rows, 30 cm apart. The plots were located at the Damascus university field in Syria.

For the season (1999-2000), 100 g of the contaminated seed were treated with acetic acid (1.22 mol) at the rate of 3 ml/100 g, lactic acid (1.22 mol) at the rate 1.5-3 ml/100 g seed and apple vinegar (containing 4 g acetic acid/100 ml) was tested at two levels 3 and 5ml/100g seed.

The fungicide "Vitavax", was used as a chemical control and was applied as recommended at a dose of 0.1 g/100g seed. The treated seed were sown

in a randomised block-trial (7.5 g seed/plot in two rows, 1.75 m/row, 30 cm spacing between rows and 60 cm between plots.
After tillering all heads were examined for bunt infection and the frequency of bunted heads calculated.

IN VITRO EXPERIMENTS

Percentage of spore germination

Acetic acid and lactic acid (1.22 mol) and vinegar at dilutions of 10-2 % and 1 % were prepared with distilled water, then autoclaved with 120 °C for 20 minutes. "Vitavax" was used as a chemical control and was diluted at three levels 0.1-0.01-0.001 %.
The spores balls were exterior sterilised with Ethanol 70 %, then crushed after dryness with sterilised needle to get completely sterilised spores, which then mixed with each dilution and then sprayed on 1.5 % water Agar media and replicated 5 times/dilution, approximately 100-200 spores per Petri dish were counted.. The Petri dishes were incubated in the dark for 5 days at 15 °C. Control was prepared by mixing spores in distilled sterilised water.

Phytotoxicity tests

- wheat seed (Cham 4) were treated with acetic acid and lactic acid at the rate 0.25 ml/100 g seed with 3 replicates of 50 seed.
- Water was added every two days to the plants to maintain adequate moisture levels.
- The wheat seed also were treated with acetic acid (1.22 mol), lactic acid (1.22 mol) and apple vinegar at the rate 3 ml/100 g seed in a second test with 4 replicates of 100 seed/replicate, according to IST International Seed Testing Association guidelines.

The treated seeds were placed in germination boxes in the folds of filter paper. Water was applied in the beginning of the test for all the treatments until the filter paper was saturated. The boxes were incubated in a growth chamber at 20 °C. Germination was determinate after 4 and 8 days after treatment. Seedling and root vitality was evaluated by measuring the shoot and root length of the first 10 seedlings for every replicate using electronic scanner (Regent instruments INC, WinRHIZO V5.Oa).

RESULTS AND DISCUSSION

IN VIVO EXPERIMENTS

A high significant reduction in the percentage of head infection with common bunt was observed in the field trials when non diluted acetic acid and lactic acid were used as a seed treatment at the rate of 0.25, 0.5 and 1 ml/100 g seed (Table 1).

Table 1: Effect of three dilutions of acetic acid, lactic acid on the control of common bunt (*Tilletia tritici* and *T. laevis*)on wheat in the field in Syria (1998-1999)

Treatments	Concentration ml/ 100g seed	% infected heads
Control		19.0
Acetic acid	0.25	0.0
	0.50	0.0
	1.00	*
Lactic acid	0.25	0.3
	0.50	0.4
	1.00	0.0

* Mistake in weeding

Acetic acid (1.22 mol) at the rate of 3 ml/100 g seed, lactic acid (1.22 mol) at the rate of 1.5-3 ml/100g seed and apple vinegar at the rates of 3-5 ml/100g seed, reduced the percentage of head infection significantly, when they were applied for control the seed borne inoculum of common bunt (*Tilletia tritici* and *Tilletia laevis*). High values of efficacy were observed for apple vinegar and acetic acid ranged 95.2-99 % at the suggested doses comparing with the seed treatment fungicide "Vitavax". In spite of significant efficacy of lactic acid against the disease, but actually it should be discarded as a promising natural alternative for control the disease because of its phytotoxicity (Table 2, Table 5).

IN VITRO EXPERIMENTS

Percentage of spore germination

Acetic acid 1.22 mol, lactic acid 1.22 mol and apple vinegar were diluted as follows, 20, 10, 2 and 1 %, pH degrees for all the dilutions were measured and gave a range 1.2-3.1. The dilutions 10, 2 and 1 % inhibited the spore germination, while using distilled sterilised water with pH=4.5 as control gave high spore germination, that emphasis the role of low acidity in suppression the spore germination of common bunt (*Tilletia tritici* and *T. laevis*) (Table 3, Fig. 1). No significant difference in spore germination of common bunt was observed among acetic acid 1.22 mol, lactic acid 1.22

mol and apple vinegar at the dilutions 10 and 2 % comparing with 0.1 and 0.01 % of the fungicide "Vitavax"(Fig. 1). Suppression of Spore germination due to spore treatment with acetic acid 1.22 mol, lactic acid 1.22 mol and apple vinegar at the dilutions 10, 2 and 1 % gave a promising ability for minimising the dose of seed treatment in the field trial, that may help to minimise the phytotoxicity of lactic acid on the germinated seedling.

Table 2: Effect of lactic acid and acetic acid (1.22 mol) and vinegar on the control of common bunt (*Tilletia tritici* and *T. laevis*) of wheat in the field in Syria (1999-2000)

Treatments	Concentration ml/ 100g seed	% infected head	Efficacy %
Non-inoculated control		0.8 B	
Inoculated control		31.5 A	
Vitavax	0.1	3.4 B	89.2
Lactic acid	1.5	4.2 B	86.6
Lactic acid	3.0	2.8 B	91.1
Acetic acid	3.0	1.5 B	95.2
Apple vinegar	3.0	0.4 B	98.7
Apple vinegar	5.0	0.3 B	99.0

Means with the same letter are not significantly different, at p≤0.05 according to Duncan's Multiple Test, n=3.

Table 3: pH degrees of lactic acid, acetic acid (1.22 mol) and vinegar dilutions

Dilutions%	Treatments			
	pH degrees			
	Lactic acid	Acetic acid	Apple vinegar	Distilled water
Basic solution	1.7	2.2	1.2	4.5
20	2.1	2.6	1.5	
10	2.2	2.7	1.9	
2	2.6	2.9	2.2	
1	2.7	3.1	2.6	

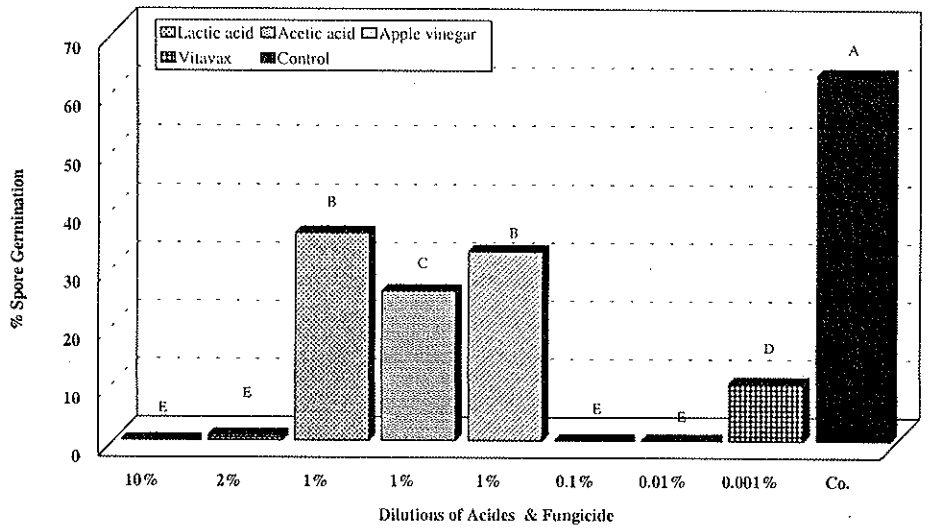

Figure 1: Effect of acetic acid, lactic acid, apple vinegar and the fungicide Vitavax on the common bunt *Tilletia tritici* and *T. laevis* spore germination. Means with the same letter are not significantly different, at p≤0.05 according to Duncan's Multiple Test, n=5.

Phytotoxicity tests

Table 4: Effect of non diluted acetic acid and lactic acid on the seed germination and on root length of wheat seedlings

Treatments	% seed germination	Seedling length/cm.	Root length/cm.
Control	79.3 A	12 A	60 A
Acetic acid	30.0 B	9 B	46 AB
Lactic acid	54.7 C	9 B	29 B

Means with the same letter are not significantly different, at p≤0.05 according to Duncan's Multiple Test, n=3.

Using of non diluted acetic acid and lactic acid as a seed treatment for control common bunt left significant reduction for the percentage of seed germination, seedling length negatively effected and a poor root system was observed due to using no diluted lactic acid for control common bunt (*Tilletia tritici* and *T. laevis*) (Table 4).

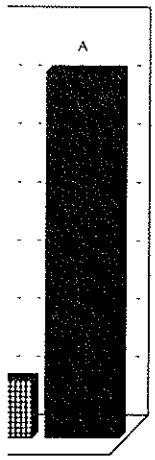

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Table 5: Effect of basic solution of lactic acid, acetic acid (1.22 mol), apple vinegar and Vitavax on the seed germination

Treatments	% seed germination	
	After 4 days	After 8 days
non-inoculated control	91.0 A	91.5 AB
Inoculated control	93.0 A	93.8 A
lactic acid	68.8 C	86.8 B
Acetic acid	85.8 B	89.5 AB
Apple vinegar	91.8 A	93.5 A
Vitavax	89.5 AB	91.3 AB

Means with same letter are not significantly different, at $p \leq 0.05$ according to Duncan's Multiple Test, n=4.

No phytotoxicity was observed on seed germination or on seedling vitality due to seed treatment with acetic acid 1.22 mol and apple vinegar after 8 days of planting comparing with inoculated and non inoculated control. On the other hand, lactic acid 1.22 mol and acetic acid caused a delay in seed germination, that was significant after 4 days and not significant after 8 days of planting, apple vinegar shows normal seed germination since after 4 days of planting (Table 5).

Table 6: Effect of basic solution of lactic acid, acetic acid (1.22 mol) and apple vinegar and Vitavax on the seedling vitality

Treatments	Seedling length/cm	Root length/cm
non- inoculated control	8 B	28 AB
Inoculated control	13 A	30 A
Lactic acid	5 C	20 C
Acetic acid	8 B	22 BC
Apple vinegar	8 B	26 ABC
Vitavax	8 B	22 BC

Means with same letter are not significantly different, at $p \leq 0.05$ according to Duncan's Multiple Test, n=4.

Safe effect of seed treatment with acetic acid 1.22 mol and apple vinegar was observed on the seedling length comparing with non inoculated control after 8 days of planting, and the same result was noticed on the root length. Significant reduction of seedling and root length was observed on the germinated plants due to use lactic acid 1.22 mol as a seed treatment comparing with non inoculated control (Table 6).

CONCLUSION

The control of common bunt is crucial for the production of quality seed. Only a small number of infected heads in the field will reduce the quality of the harvested wheat, because of the stench of the bunt spores.

Treatments based on pH-effects by manipulation the pH-value on the seed surface have been known for a long time (Buttress and Dennis, 1947). The effect of lime as a seed treatment against common bunt is likely to be an effect of water reaction (pH) since lime is a strong base. While acetic acid is a natural substances with a very low acidity and has a low oral toxicity to humans in the same time. 5 % solution of acetic acid at a dose of 20 ml/kg has reduced common bunt (*Tilletia tritici*) in winter wheat by 91.5 % when applied as a seed treatment. While the experiment with spring barely shows that barely leaf stripe (*Pyrenoghora graminea*) can be significantly reduced the infection 55.9 % when 5 % solution of acetic acid applied as a seed treatment in a dose of 40 ml/kg. And the negative effect on seed vigour seems to be proportional to the dose applied, that start to appear when more than 40 ml/kg was applied (Borgen and Kristensen, 2001).

In the present test, seed treatment with the commercial product of acetic acid and lactic acid were highly effective against the seed borne inoculum of common bunt on wheat. Phytotoxicity however was observed due to seed treatment with both products.

Seed treatment with 1.22 mol of acetic acid and lactic acid also reduced significantly the percentage of infected heads in a field experiment. Dilutions of acetic and lactic acid 10, 2 and 1 % led to significant inhibition of spore germination in vitro. Efficacy of acetic acid and lactic acid was attributed to the low acidity of these acids and their dilutions (Table 3). Seed treatment with acetic acid 1.22 mol gave effective control and no phytotoxicity, high levels of control by lactic acid 1.22 mol was accompanied by poor seed germination and reduced seedling vitality.

High levels of disease control with no harmful side effects on seed germination and seedling vitality was observed when apple vinegar was applied as a seed treatment for the control of the seed borne inoculum of common bunt.

Dilutions of apple vinegar 10, 2 and 1 suppressed common bunt germination in vitro. Inhibition was probably due to the low acidity of apple vinegar.

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