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# Organic seed-treatment as a substitute for chemical seed-treatment to control common bunt of wheat

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## Abstract

Common bunt of wheat, caused by *Tilletia laevis* and *T. tritici*, is a major seed and soil-borne disease in West Asia and North Africa. The use of resistant cultivars and chemical seed-treatments are the current control measures used to combat this disease. The aim of this study was to investigate alternative control measures to chemical seed-treatments that are environmentally friendly to support cultivar resistance. Several organic nutrients [skimmed milk powder, hucket (local skimmed milk) and wheat flour] at a concentration of 160 g per kg of seeds were used as seed-treatments on two susceptible bread and durum wheat cultivars (Bau and Sebou, respectively) to examine their effectiveness in controlling the disease. Field trials over four years showed that skimmed milk powder, hucket, and wheat flour reduced common bunt infection levels by 96%, 93% and 62%, respectively. In most cases, the effectiveness of the skimmed milk powder and hucket was equal to the chemical seed-treatment; thus, these organic nutrients offer an effective and environmentally safe alternative to chemical treatments. However, a study of their economic value as well as of their effect on seed germination, and field emergence is needed.

## Introduction

Common bunt, caused by *Tilletia laevis* Kühn [*T. foetida* (Wallr.) Liro.] and *T. tritici* (Bjerk.) Wint. [*T. caries* (DC) Tul.], is the most widespread smut of wheat in countries of West Asia and North Africa (i.e. WANA region) (Saari et al., 1996). In the majority of these countries, the most prevalent common bunt pathogen is *T. laevis* (Saydam et al., 1972; Finci, 1981; Finci et al., 1983; Mamluk et al., 1990; Mamluk and Zahour, 1993; Ismail et al., 1995). Actual losses are not well documented, but yield losses of 1–7% are common in WANA countries and can occasionally be severe (Mamluk, 1992). Losses as high as 10–20% and 25–30% have been recorded in Turkey (Yüksel et al., 1980; Parlak, 1981) and Iran (Bamdadian, 1993), respectively. In addition to the direct loss of grain yield, the bunt reduces grain quality (Williams, 1983), even at levels as low as of 0.05% by weight (Anonymous, 1984).

Although the pathogens causing common bunt are soil-borne, the main source of infection seems to be through contaminated seeds. The natural level of contaminated seed can be as high as $230 \times 10^3$ spores per wheat kernel (O.F. Mamluk, unpubl.).

Common bunt is controlled by the use of resistant cultivars (Mamluk and Nachit, 1994; Rubiales et al., 1996), and chemical seed-treatment (Hoffmann and Waldher, 1981; Line, 1993). Hoffmann (1982), however, estimated that less than 40% of the seed sown in the WANA region is treated. Costs of chemical seed-treatment and distribution of treated seed are major constraints. In low-input agriculture with wheat or barley as sole crops, farmers do not use certified and treated seed for every planting season, nor do they have seed-treatment facilities. Furthermore, loss of efficacy of registered fungicides for seed-treatment to control common bunt has been observed in some years and locations (Gaudet and Puchalski, 1989). In recent years, the trend in agriculture towards greater sustainability and public concern for hazards associated with the use of chemical pesticides has increased (Jacobsen and Backman, 1993); hence there is a growing need to look for alternative control measures to complement varietal resistance.

The use of organic nutrients as seed-treatments to control the common bunt pathogen in Europe, *T. tritici*, showed promising results (Becker et al., 1990; Becker and Weltzien, 1993; Winter et al., 1994; Borgen et al., 1995; Borgen, 1997; Winter et al., 1997). However, no efficacy data on these organic nutrients for controlling either *T. laevis*, the most prevalent common bunt pathogen on bread and durum wheat in WANA region (Mamluk and Zahour, 1993) or both pathogens are available.

The aim of this study was to determine if seed-treatment by organic nutrients is effective under the dry conditions of the Mediterranean environment against the two common bunt pathogens (*T. tritici* and *T. laevis*) together and separetely.

## Materials and methods

A four-year field test was conducted at the International Center for Agricultural Research in the Dry Areas (ICARDA) main research station, Tel Hadya, Syria during 1994/95, 1995/96, 1996/97, and 1997/98 seasons. The soil at the station is a fine clay montmorillonitic, thermic, calcixerollic xerochrept, and representative of considerable areas of the Mediterranean basin. Two cultivars susceptible to both pathogens of common bunt (*T. tritici* and *T. laevis*) were used, Bau (bread wheat) and Sebou (durum wheat). Inoculum used was a bulk of teliospores collected annually from naturally infected spikes in Syria. Inoculation was done by rubbing the teliospores of both pathogens in a ratio of 1 : 1 or of each pathogen alone with the seed by hand for 3 min at a rate of 6 mg bunt spores per 1 g of seed (ca. $80 \times 10^3$ spores per seed). Each cultivar was divided into 9 g batches and treated with one of the following treatments: Vitavax-200 (carboxin 200 g per l, thiram 200 g per l, Uniroyal Chemical Co.); skimmed milk (Frema reform, Germany); hucket (local skimmed milk) and wheat flour. The chemical compound was applied at a rate of 2 ml per kg of seeds and the organic nutrients at 160 g + 100 ml of water per kg of seeds. Hucket and wheat flour treatments were included only in 1996/97 and 1997/98 seasons. However, the other treatments were applied for the four seasons consecutively. All treatments were prepared in distilled water. Each treatment was added to seed contained in a glass flask. Then, seeds were agitated to distribute the respective solutions uniformly over the seed surface and allowed to air dry on filter paper at room temperature. Untreated, inoculated seeds with the two pathogens, as well as with each pathogen alone, were included as controls for each cultivar and in each replicate.

To check the soil contamination, seeds for each cultivar were surface-sterilized in a 0.5% sodium hypochlorite solution for 5 min, rinsed several times with sterile distilled water and air dried on filter paper. The trials were planted on December 12, 22, 31, and 15 in 1994, 1995, 1996, and 1997, respectively. The plots, each consisting of two-rows 1 m long, were arranged in a randomized complete block design with three replications. At maturity, half of the plots were harvested and the percentage of bunted spikes was determined by counting healthy and bunted spikes. A plant was considered bunted if a spike contained at least one bunt ball.

All data were subjected to analysis of variance using a Generalized Linear Model (Genstat 5 for Windows, release 4.1) with binomial error distribution and logit link, which accounted for year, cultivar, pathogen, seed-treatment, and all possible interactions involving these treatments. Duncan's multiple range test was used for mean separation ($P \leq 0.05$) for all treatments.

## Results and discussion

All main effects and the interaction effects were highly significant at $P < 0.001$, except cultivar effect, year × cultivar, and year × seed-treatment × cultivar interactions (Table 1). Bunt incidence in the disinfested, uninoculated, and untreated controls was less than 2% in all years, indicating negligible infection from the soil (Table 2). In the inoculated, untreated checks, infection levels ranged widely from 10.8% to 88%, but most were over 50%. Differences in disease incidence were noted between the bread and durum wheat cultivars, but were not significant. In general, the bread wheat cultivar 'Bau' was more susceptible to common bunt than the durum wheat cultivar 'Sebou' (Table 2). The mean bunt levels for Bau and Sebou in inoculated, untreated

*Table 1.* Analysis of variance for effect of seed-treatments on the control of common bunt of wheat

| Source of variation | d.f | Variance | Mean variance | *P* |
|---|---|---|---|---|
| Year | 3 | 413.5 | 137.8 | < 0.001 |
| Cultivar | 1 | 3.9 | 3.9 | 0.048 |
| Pathogen | 3 | 1342.9 | 447.6 | < 0.001 |
| Seed-treatment | 4 | 8821.6 | 2205.4 | < 0.001 |
| Pathogen × Seed-treatment | 8 | 34.9 | 4.4 | < 0.001 |
| Pathogen × Cultivar | 3 | 163.3 | 54.4 | < 0.001 |
| Seed-treatment × Cultivar | 4 | 53.8 | 13.4 | < 0.001 |
| Pathogen × Seed-treatment × Cultivar | 8 | 62.5 | 7.8 | < 0.001 |
| Year × Cultivar | 3 | 9.6 | 3.2 | 0.022 |
| Year × Pathogen | 9 | 113.1 | 12.6 | < 0.001 |
| Year × Seed-treatment | 8 | 65.5 | 8.2 | < 0.001 |
| Year × Pathogen × Seed-treatment | 14 | 76.5 | 5.5 | < 0.001 |
| Year × Pathogen × Cultivar | 9 | 39.2 | 4.4 | < 0.001 |
| Year × Seed-treatment × Cultivar | 8 | 21.6 | 2.7 | 0.006 |
| Year × Pathogen × Seed-treatment × Cultivar | 14 | 95.2 | 6.8 | < 0.001 |
| Residual | 199 | 640.8 | 3.2 | |
| Total | 298 | 11958.2 | 40.1 | |

plots were 75% and 57%, respectively over years. *T. laevis* alone was less virulent on both varieties than *T. tritici* alone or when both pathogens were inoculated together. This might be due to the adaptation of these cultivars to *T. laevis*, as it is the most prevalent common bunt pathogen in WANA countries (Mamluk and Zahour, 1993; Ismail et al., 1995). The bunt incidence was higher in 1994/95 than in any other season. This was probably due to the lower soil and air temperatures in this season compared to other seasons. The mean soil and air temperatures in 1994/95 for the 10 days after planting ranged between 6–9 °C and 4–9 °C, respectively, compared to 8–12 °C and 6–13 °C for the other seasons (Meteorological station, T. Hadya, ICARDA). Seeding into cool soils increases the incidence of the disease because the relatively low optimal temperature for spore germination and seedling infection by the bunt fungus is coupled with the concomitant slow development of the wheat plant (Holton and Heald, 1941). Moreover, the strongest attack by common bunt was observed when the daily mean temperature during days 1–11 after planting is 6–7 °C (Johnsson, 1992).

The main objective of this study was to evaluate a few organic nutrient seed-treatments that have

*Table 2.* The effect of organic seed-treatments (skimmed milk, hucket, and flour) on the control of common bunt of wheat (*Tilletia tritici* and *T. laevis*) as compared to chemical seed-treatment (Vitavax-200); seasons 1994/95–1997/98

| Treatment | % of head infection | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1994/95 | | 1995/96 | | 1996/97 | | 1997/98 | |
| | Bau[1] | Sebou | Bau | Sebou | Bau | Sebou | Bau | Sebou |
| *T. tritici* and *T. laevis* (check) | 88.0 a[2] | 84.5 a | 83.4 a | 57.0 a | 48.7 a | 36.5 a | 81.6 a | 52.6 ab |
| *T. tritici* and *T. laevis* + Vitavax-200 | 2.4 c | 0.7 cd | 9.5 b | 3.1 b | 0.0 e | 0.9 efg | 3.2 fg | 1.8 efg |
| *T. tritici* and *T. laevis* + skimmed milk | 9.7 b | 5.6 b | 2.1 c | 3.0 b | 0.7 e | 0.4 fg | 2.2 gh | 4.6 de |
| *T. tritici* and *T. laevis* + hucket | n.t.[3] | n.t. | n.t. | n.t. | 1.7 e | 4.3 cd | 7.0 ef | 6.3 d |
| *T. tritici* and *T. laevis* + flour | n.t. | n.t. | n.t. | n.t. | 12.8 c | 10.0 bc | 31.9 bc | 46.1 abc |
| *T. tritici* (check) | n.t. | n.t. | 56.3 a | 69.2 a | 35.9 ab | 39.9 a | 55.8 ab | 63.4 a |
| *T. tritici* + Vitavax-200 | 0.9 c | 0.0 d | 0.7 c | 4.2 b | 0.3 e | 2.6 def | 0.6 hi | 0.2 g |
| *T. tritici* + skimmed milk | 7.3 b | 6.2 b | 1.2 c | 5.0 b | 0.2 e | 2.6 def | 0.0 i | 4.1 de |
| *T. tritici* + hucket | n.t. | n.t. | n.t. | n.t. | 0.7 e | 2.1 defg | 0.6 hi | 1.3 efg |
| *T. tritici* + flour | n.t. | n.t. | n.t. | n.t. | 4.6 d | 21.1 ab | 22.3 cd | 19.3 c |
| *T. laevis* (check) | n.t. | n.t. | 68.4 a | 43.1 a | 21.2 bc | 10.8 bc | 34.8 abc | 20.9 bc |
| *T. laevis* + Vitavax-200 | 0.7 c | 0.0 d | 0.0 c | 1.8 b | 0.0 e | 0.0 g | 0.9 hi | 1.0 fg |
| *T. laevis* + skimmed milk | 0.8 c | 2.8 bc | 1.5 c | 1.7 b | 0.0 e | 0.0 g | 1.8 gh | 0.5 g |
| *T. laevis* + hucket | n.t. | n.t. | n.t. | n.t. | 0.9 e | 0.0 g | 1.7 gh | 4.8 def |
| *T. laevis* + flour | n.t. | n.t. | n.t. | n.t. | 11.6 c | 2.3 def | 12.9 de | 3.6 def |
| Disinfected seed | 0.0 c | 0.0 d | 1.5 c | 0.9 b | 0.0 e | 0.0 g | 0.6 hi | 2.2 efg |

[1]Bau = bread wheat; Sebou = durum wheat.
[2]Means followed by the same letter in each column are not significantly different at $P = 0.05$ according to Duncan's multiple range test. The test was performed on logit transformed values.
[3]n.t. = not tested.

the ability to control common bunt seed-borne phase with effects comparable to chemical fungicides. To date, only few studies have dealt with this subject, all of them described only the control of these organic nutrients against *T. tritici* alone. All seed-treatments reduced significantly common bunt infection levels in all years for both cultivars and pathogens (Table 2). A 99–100% reduction in the infection level for both cultivars was obtained with Vitavax-200 and skimmed milk in 1996/97 when seeds were inoculated with both pathogens (*T. laevis* and *T. tritici*) and with *T. laevis* alone. The average reduction in bunt infection levels over years and cultivars was 97%, 96%, 93%, and 62% for Vitavax-200, skimmed milk, hucket, and wheat flour, respectively. The results showed that the effect of skimmed milk and hucket seed-treatment were consistent over seasons and against both common bunt pathogens. In general, the reduction of common bunt infection by skimmed milk and hucket seed-treatment was as efficient as the chemical seed-treatment. The wheat flour was less effective, but still resulted in a marked reduction of bunt infection. Similar results were obtained by Becker et al. (1990), and Becker and Weltzien (1993), where skimmed milk seed-treatment reduced common bunt infection by 97%. These workers, however, found a much higher reduction in bunt infection (93% and 96%, respectively) with the wheat flour seed-treatment than was found in this study (62%). This might be due to the use of a different wheat flour product and to the higher concentration of the treatment used in their studies (400 g of wheat flour per kg of seeds). In general, the dosage of these organic nutrients can affect seed germination, especially skimmed milk (Becker and Weltzien, 1993; Borgen, 1997; Winter et al., 1997). Higher dosages of skimmed milk, (400 g per kg of seed) can reduce the germination; however, yields are not depressed because good bunt control is achieved (Becker and Weltzien, 1993).

The reduction of common bunt infection by these organic nutrients might be related to an increase of the antagonistic potential of the soil-borne microorganisms, or to the production of toxic metabolites, which might have direct inhibition of teliospore germination. Becker and Weltzien (1993) found that skimmed milk powder and wheat flour enhanced soil-borne antagonistic microorganisms, especially *Bacillus*, *Pseudomonas* spp., yeast and several species of the fungal genera *Mucor* and *Rhizopus* on the seed surface. Moreover, they observed a direct inhibition of *T. tritici* chlamydospore germination by these nutrients.

Few reports have dealt with the biological control of common bunt. Some strains of *Pseudomonas fluorescens* proved to inhibit *T. laevis* teliospore germination and reduce common bunt incidence by 65% when wheat seeds were treated with these strains (McManus et al., 1993). Kollmorgen and Jones (1975) showed that isolates of *Streptomyces* and *Bacillus* species can cause marked reductions in chlamydospore germination of both *Tilletia* species *in vitro*. Kollmorgen (1976) found that *Bacillus* species reduced disease incidence of common bunt under field conditions. Becker et al. (1990) reported that some *Gliocladium* species reduced common bunt incidence considerably. Hokeberg et al. (1997) and Johnsson et al. (1998) found that one *Pseudomonas chlororaphis* isolate, MA 342 strongly suppressed *T. tritici* in the greenhouse and in the field, respectively.

In conclusion, the skimmed milk powder and hucket proved to be very effective in controlling common bunt infection caused by the two pathogens *T. laevis* and *T. tritici* in a Mediterranean environment. The cause of decreased common bunt infection through the seed-treatment with organic nutrients and the mechanism of this control is not known, but studies to address this aspect are in progress. Future studies should also include the effect of the skimmed milk powder and hucket on seed germination, field emergence, and grain yield as well as on the application technique and economic return.

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