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**Research Article**

# Effects of physical state of formulations on the potential of *Trichoderma harzianum* 199 against wheat common bunt

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**Abstract:** Potential of three physically different formulations of biocontrol agent *Thrichoderma harzianum*199 was investigated in a field trial against wheat common bunt caused by *Tilletia laevis* Kühn. Wheat seeds of cv. Chamran were treated with liquid, semi-solid (gel mixed suspension) and solid (talc powder) formulations prior to planting. Field practices were performed based on Khuzestan wheat planting schedule and no chemical was used until harvesting. The results of analyzed data showed significant effect of formulation type on common bunt incidence. Tetraconazole (chemical check) showed common bunt reduction (97.7%) and among bio-formulations, gel-mixed suspension was significant in disease reduction (43.41%), but it was next to talc and liquid formulation in yield, harvested weight, healthy spikes and stem height. Talc formulation reduced bunt infection (39.07%) and showed better than gel mixed suspension in yield and some yield components. Conversely, liquid formulation enhanced bunt incidence (25.31%) but was almost same as the talc and better than gel formulation in yield and yield components. General findings of this experiment indicate that physical form of *T. harzianum* 199 formulation can effectively influence both common bunt prevention and agronomic potential of Chamran wheat cultivar.

**Keyword:** biological control, biofungicide, formulation, *Tilletia laevis*, organic wheat

## Introduction

*Trichoderma* spp. are cosmopolitan saprophytic free living soil inhabitants and plant growth promoters, capable of synthesizing antagonistic compounds and inhibiting phytopathogens directly or via inducing plant resistance responses (Harman *et al*., 2004; Sharma *et al.,* 2012; Sargin *et al.,* 2013). *Trichoderma* is a potent biocontrol agent extensively applied in various soil-borne and foliar plant disease management programs through several delivery methods (Verma *et al.,* 2007; Kumar, 2013). Formulation is the blending of active biological ingredients with inert carriers in order to improve its physical characteristics (Kumar, 2013). It profoundly influences many aspects of a biopesticide product such as shelf life, safety and antagonistic capability (Burges, 1998; Warrior, 2002; Bertolini and Pratella, 2003). Different formulations of *T.harzianum* and other fungal biocontrol agents have been produced by means of solid substrate fermentation (SSF) or liquid fermentation (LF) procedures (Fravel, 2005). They comprise plant based formulations (Rao *et al*., 1998), powder and

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pelet based formulations (Küçük and Kivanc, 2005; Jayaraj *et al*., 2006; Bhat *et al*., 2009; Martı´nez-Medina *et al*., 2009), liquid based formulation consisting of oil based (Ahamed, 2011) and non oil based liquids (Taylor *et al*., 1991), paste and gel formulations (Chen-Fu and Wen-Chien, 1999; Łukanowski, 2006; Jayaraj *et al*., 2006) alginate capsules (Lewis and Papavizas, 1985; Shaban and El-Komy, 2001) bioplastic granules (Accinelli *et al*., 2009), and chitin fortified formulations (Solanki *et al*., 2012), which according to their physical forms can simply be placed in three separate categories including liquid, semi-solid and solid states.

*Tilletia laevis* Kühn [*T. foetida* (Waller.) liro] is the main causal agent of wheat common bunt in Iran and some parts of the world (Ershad, 1977; Wilcoxson and saari, 1996). Common bunt potentially is a very serious problem in organic wheat production (Borgen, 2004). The greatest epidemics of bunt have been reported after Second World War from Central Europe and in some regions of Poland (Lukanowsky, 2006). Owing to high efficacy of chemical seed treatment, breeding for common bunt resistance has been long disregarded and many commercial cultivars are susceptible to bunt now (Liatukas and Ruzgas, 2009). It is a major seed and soil borne disease in west Asia and North Africa (El-Naimi *et al.,* 2000). High humidity and low temperature in soil support teliospore germination and development of dicaryotic infectious hyphae which are involved in seedling infection intracellularly and then in spike and young kernels intercellularly leading to formation of bunt balls replete with fungal teliospores (Agrios, 2005).

Among wheat diseases, bunt and smut are ranked in the second place of importance in west Asia (Mamluk and Zahour, 1993). In Iran, the most infected wheat fields are located in northern and northwestern parts and *T. leavis* is predominant species in the country (Sharifnabi and hedjaroud, 1992). Losses of bunt are estimated at 5-7 percent in west Asia (Hoffmann, 1982), 10-20% in Turkey (Yüksel *et al.,* 1980; Parlak, 1981) and 25-30% in some parts of Iran (Bamdadian, 1993). Triazoles are effective chemical fungicides for seed protection, causing disruption in ergosterol biosynthesis pathway in fungal target cells thereby controlling many species and strains of fungi (Ghannoum and Rice, 1999; Vanden Bossche *et al*., 1990).

At the present time, the number of control measures acceptable in organic wheat production is limited. Various organic material and bioformulations have been used for wheat seed biotreatment (El-Naimi *et al.,* 2000). One possible substitute for harmful chemicals could be the use of safe and effective biocontrol agents like *Trichoderma* (Nielsen *et al.,* 2000). Seed biopriming with *Trichoderma* species induces deep positive changes in plant physiological characteristics (Entesari *et al.,* 2013). Seed treatment with *T. viride* or in combination with Vitavax has proved a promising approach for wheat root rot control caused by *Drechslera biseptata* and *Fusarium moniliforme*. (Amira and Amal, 2008). Liquid preplant wheat biotreatment with *T. viride* ($5 \times 10^6$ spore/ml) resulted in lower bunt infestation same as sulphur treatment effect (Lukanowsky, 2006). No favorable outcome from liquid treatment of *Trichoderma* spp. was observed when used against wheat common bunt, while mustard (*Sinapis alba*) flour showed acceptable control (Mehrabi *et al.,* 2009).

The present research attempts to find a biological substitute for economically expensive and ecologically harmful chemical fungicides by scrutinizing possible effect of physical properties of biotreatments on bunt reduction and taking an important step toward organic wheat farming in Iran.

## Materials and Methods

### Microorganism and inoculum preparation

*T. harzianum* 199 obtained from Iranian Research Institute of Plant Protection was used in this study. The fungus was first cultured on PDA (potato dextrose agar) and incubated at 27 °C until abundant conidia production was observed. Sterile distilled water was poured on plate surfaces, conidia were scraped and released in the water with a spatula and passed through folded cheesecloth two times, the final spore suspension gathered in an Erlenmeyer flask was adjusted with sterile water to give a spore concentration of $10^7$ conidia per milliliter using a haemocytometer. All the procedure was carried out under aseptic condition (modified from Abdel-Kader *et al*, 2012). This suspension was used for preparing experimental formulations.

**Liquid formulation**

Liquid formulation was prepared by adding 1 g/l (w/v) of CMC (Carboxy methylcellulose) to needed volume of conidial suspension.

**Talc powder formulation**

Equal parts volume to weight (v/w) of conidial suspension from stock was added to sterile talc powder, mixed well manually and dried under sterile condition, the resulting product was then ground to fine dust (modified from Subash *et al*, 2013).

**Gel mixed suspension formulation**

Fully covered culture plates of *T. harzianum*199 were washed and cleaned of fungal growth with sterile distilled water and then a volume of washed gel was added to equal volume of spore suspension from source (amended with 1% CMC) and mixed in a blender at low rotational mode for 30 second (modified from Jayaraj *et al*, 2004).

**Chemical seed treatment**

Tetraconazole (lospel®125g/l), was used at concentration of 1 ml/kg (chemical check).

**Field operations**

Planting procedure was carried out on December 20 and no chemical was used throughout the growing period. The experiment was conducted based on randomized complete block design and replicated thrice at Ahvaz Agricultural Research Station of Khuzestan Province in 2013. Seeds of Chamran wheat cv. were coated via soaking or rubbing. Treatments of the experimental trail were:

$T_1$ -check (seeds without treatment)

$T_2$ -check (seeds contaminated with *T. leavis* (5 g/kg)

$T_3$ -check (seeds inoculated with *T. harzinum* ($10^7$ conidia/ml)

$T_4$ -liquid formulation treatment ($10^7$ conidia /ml)

$T_5$ -talc formulation treatment ($10^7$ conidia /g)

$T_6$ -gel-mixed formulation treatment ($0.5 \times 10^7$ conidia/ml)

$T_7$ -Tetraconazole (1 ml/kg) as chemical treatment.

*T. laevis* pre-contamination was fulfilled externally in rubbing mode for $T_2$, $T_4$, $T_5$, $T_6$ and $T_7$ at the proportion of 5g teliospores/kg seeds. Plot size was 6 $m^2$ (1.5 × 4) with 6 planting row.

Disease incidence which is equal to yield loss for common bunt was calculated based on the following formula (Cooke, 2006).

Disease Incidence = (Number of infected plant units / total number of plants assessed) × 100

**Sampling method and statistical analysis**

At the complete spike maturity state, in mid May 2013, a half square meter quadrate was used for sampling from center of the plots. Number of infected spikes and related important yield components for each treatment were recorded and the resulting data processed statistically using Minitab and MSTATC. Significant differences were tested by one-way analysis of variance (ANOVA) and means were compared by Duncan's multiple range test after ANOVA at $p < 0.05$.

**Results**

Although some traits including thousand grain weight, spike grain weight and yield were not significant (Table 1).

**Table 1** Summery of analysis of variance of the traits based on mean square.

| Source of variation | df | Infected spikes | Percent infection | Grain yield | Stem length | Healthy spikes | Harvested mass | Thousand grain weight |
|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 0.190 | 73.722 | 519.048 | 49.476 | 80.190 | 5392.190 | 6.392 |
| Treatment | 6 | 23.778 | 631.694 | 628.746 | 77.111 | 2813.317 | 14589.079 | 10.065 |
| Error | 12 | 1.607 | 173.288 | 308.937 | 19.754 | 767.079 | 4369.079 | 5.950 |
| Significance | | ** | * | ns | * | * | * | Ns |

* and ** refer to significant differences at $P < 0.05$ and $P < 0.01$, respectively, ns: non-significant.

**Table 2** Effects seed treatment by different formulations of *Thrichoderma harzianum*199 on disease incidence and yield of wheat caused by *Tilletia laevis* under field condition.

| Treatments | Bunted spikes (%) | Disease incidence (%) | Harvested weight | Healthy spikes | Yield | Spike grain weight | Thousand grain weight | Stem height |
|---|---|---|---|---|---|---|---|---|
| Non-contaminated check ($T_1$) | 0c | 0c | 249.3ab | 170.0a | 81.00 | 0.8140 | 35.20 | 48.33b |
| Tetraconazole ($T_2$) | 0.67c | 0.64c | 238.0ab | 130.0ab | 73.33 | 0.9408 | 38.53 | 55.67ab |
| Trcichoderma check ($T_3$) | 3.00bc | 1.96bc | 283.3ab | 148.7ab | 92.67 | 0.9477 | 38.07 | 59.53ab |
| Gel-mixed formulation ($T_4$) | 21.67ab | 16.42ab | 212.0b | 107.7b | 56.33 | 0.8953 | 37.00 | 56.00ab |
| Talc formulation ($T_5$) | 28.33a | 17.69ab | 312.7ab | 132.0ab | 85.00 | 0.8658 | 33.30 | 58.33ab |
| Liquid formulation ($T_6$) | 43.33a | 36.38a | 330.7ab | 119.0ab | 77.67 | 0.8890 | 37.97 | 62.67a |
| Contaminated check ($T_7$) | 63.33a | 29.03a | 418.7a | 150.7ab | 101.70 | 0.8994 | 36.97 | 63.32a |

Values in each column followed by different letters are significantly different (Duncan's multiple range test, $P < 0.05$).

**Effects of formulations on analyzed traits**

**1-Bunted spikes**: Contaminated check showed the highest number of infected spikes ($T_2$), followed by liquid formulation ($T_4$), while the lowest belonged to $T_7$, $T_1$ and $T_3$ respectively. Among biotreatments, gel mixed formulation ($T_6$) reduced bunted spikes significantly (Table 2).

**2-Disease incidence:** $T_4$ (liquid formulation) showed more disease incidence than contaminated check ($T_2$) while both fall in the same class. Healthy check and Tetraconazole showed lowest disease incidence and among bioformulations as anticipated, gel mixed suspension ($T_6$) showed better bunt reduction (Table 2).

**3-Harvested mass**: contaminated check ($T_2$) showed highest harvested mass (a) and the lowest was observed in gel mixed formulation.

**4-Grain weight**: $T_3$ and $T_7$ showed higher grain weight per spike while the $T_1$ was the lowest. Among biotreatments Gel mixed suspension ($T_6$) showed better performance.

**5-Thousand grain weight**: $T_7$ and $T_3$ showed higher thousand grain weight, the lowest belonged to talc formulation ($T_5$), liquid formulation showed better than other biotreatments.

**6-Healthy spikes**: The highest infected and non infected spikes were observed in contaminated check ($T_2$) followed by liquid formulation ($T_4$) (c). Unexpectedly the lowest was observed in non- contaminated check.

**7-Yield**: the highest yield was observed in ($T_2$) and ($T_3$). (T6) although the best in disease reduction it showed the lowest yield among the biotreatments.

**8-Plant height**: The highest plant length was observed in ($T_2$) and ($T_4$). For this trait ($T_1$) was the lowest. Among biotreatments, gel mixed formulation showed lower stem height than did talc and liquid treatments.

## Discussion

Improving physical structure and bioactive capability of biofungicides through the best possible formulation have recently become the goal for biopesticide industry and organic agriculture. Subsequent to seed biopriming, *Trichoderam* spp. can be well established in different pathosystems and reduce plant diseases and alleviate abiotic and physiological stresses in seed and seedlings (Harman *et al.,* 2004; Mastouri *et al.,* 2010). In this research *T. harzianum* 199 was prepared in three physical shapes (liquid, semi-solid, solid) in the form of liquid suspension, gel mixed suspension and powder and was evaluated against wheat common bunt in the field. Semi solid gel mixed suspension which is an innovation initiated in this research, proved effective in disease reduction (43.4 %) compared with contaminated check, but fell behind talc and liquid formulations in traits like harvested weight, healthy spikes, yield and stem height (Table 2). It seems that, better bunt control of this formulation was due to direct contact of *T. laevis* teliospores with hydrolytic enzymes and antibiotics present in the gel, resulting in less infected spikes and disease incidence. No comparison is possible in this regard, because such a semi solid matrix treatment has not earlier been reported for wheat. An almost similar shape of formulation (gelatinized corn starch) prepared by cooking corn starch in distilled water applied for corn seed coating against *Fusarium verticillioides* showed less effective than talc formulation but more effective than paddy husk, and wheat bran formulations (Nayakaa *et al.,* 2008). Unfavorable effect of gel mixed suspension on yield, harvested weight and healthy spikes (Table 2) can be attributed to incompatibility of biocontrol strain or harmful effects of gel metabolites, because in general proper biopriming with strains of *Trichoderma*improves seedling emergence, leaf area and dry weight (Kleifeld and Chet, 1992). There is a strongly identical result (39.07% disease reduction) from talc based formulation in this research with the work of Mehrabi *et al*. (2009) in which powder coated seed with *T. harzianum* reduced bunt 32.8% while *T. koningii*, T. *brevicompactum* and *T. virens* reduced disease 29.4%, 29.9%, 36.3%, respectively. Although yield components of talc formulation treatment were lower than those of contaminated check, there is a report that rice seed treatment with *Trichoderma viride* talc formulation enhanced crop growth, grain yield, root and shoot lengths, dry weight, plant height and reduced sheath blight comparable to the treatment with Carbendazim (Karthikeyan *et al.,* 2005). In some cases no differences have been observed between solid or liquid formulations. For example seeds treated by *T. harzianum* either through liquid or powder coating formulations both showed the same efficiency against *Pythium* spp. in infested soils (Taylor *et al.,*1991). While in this research liquid treatment failed totally in common bunt prevention and not only didn't reduce the disease but also there was 25.31% increase in disease incidence and this is contrary to the report of (Lukanowsky, 2006) that liquid biotreatment by *Trichoderma viride* ($5 \times 10^6$ per/ml) decreased common bunt (18.2%) same as sulphur treatment. An unexpected response to contamination with common bunt disease was observed in contaminated check in which the number of healthy spikes, yield, and the remaining favorable agronomical traits except thousand grain weight were better than all the treatments. Justification for this phenomenon can be sought in natural response of wheat to the presence of the common bunt pathogen which causes more tiller and spike production in some wheat varieties. Increased tiller number has been reported for three wheat varieties including Aranka (2%), Munk (18%), Vinjett (24%) after inoculation with *Tilletia* (DumalasoVá and Bartos, 2007). Low temperature at the beginning of tillering stage may have influenced the development of *Tilletia*, and hampered the spike infection. It has been reported that, two weeks of continuous low temperature prompts plentiful tillering and healthy spikes of tillers in infected plants (Zscheile, 1955). Some 30% of spikes on infected plants can escape from disease and produce healthy spikes (Murray and Wright, 2007) which can explain higher yield in contaminated check. On the other hand, there are reports that incompatible strains of biocontrol capable of producing harzianic acid (Vinale *et al*., 2009) or trichothecin (Marfori *et al*., 2003) can have adverse effect on different parts of the plants including root, shoot and seedlings, which explains why yield components were lower than expectations in biological treatments. General findings of this research indicate that in addition to disease control potential, the biological seed treatment with *Trichoderma* requires information about compatibility of biocontrol strain and wheat cultivar.

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## References

Abdel-Kader, M. M., El-Mougy, N. S., Aly, M. D. E. and Lashin, S. M. 2012. Long activity of stored formulated bio-agents against some soil borne plant Pathogenic fungi causing root rot of some vegetables. Journal of Applied Sciences Research, 8 (4): 1882-1892.

Accinelli, C., Saccàa, M. L., Hamed, K. A., Zablotowiczc, R. M. and Wilkinsond, R. 2009. Use of a granular bioplastic formulation for carrying conidia of a non-aflatoxigenic strain of *Aspergillus flavus*. Journal of Bioresource Technology, 100: 3997-4004.

Agrios, G. N. 2005. Plant Pathology, Fifth Edition. Academic Press, New York.

Ahamed, M. V. 2011. Studies on production of oil based formulation of *Trichoderma* and their efficacy on foliar diseases of groundnut. MSc degree. University of Agricultural Sciences, Dharwad (Institute).

Amira, M. A. and Amal, A. A. 2008. Evaluation of antifungal activity of vitavax and *Trichoderma viride* against two wheat root rot pathogens. Journal of Applied Biosciences, 6: 140-149.

Bamdadian, A. 1993. A short report on the wheat bunt in Iran. Plant Protection Research Institute, Evin, Iran. 2 pp.

Bertolini, M. P. and Pratella, G. C. 2003. Non-conventional methods for the control of post-harvest pear diseases. Journal of Applied Microbiology, 94: 761-766.

Bhat, K. A., Anwar, A., Lone, G. M., Hussain K. and Nazir, G. 2009. Shelf life of liquid fermented product of *Trichoderma harzianum* in talc. Journal of Mycology and Plant Pathology, 39 (2): 263-265.

Borgen, A. 2004. Control of seed borne diseases in organic seed propagation. In: Proceedings of the First World Conference on Organic Seed “Challenges and Opportunities for Organic Agriculture and the Seed Industry”. July 5–7 FAO Headquarters, Rome. Italy, 170-171.

Burges, H. D. 1998. Formulation of microbial pesticides. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Chen-Fu, C. and Wen-Chien, L. 1999. Formulation of a biocontrol agent by entrapping biomass of *Trichoderma viride* in gluten matrix. Journal of Bioscience and Bioengineering, 6: 822-824.

Cooke, B. M. 2006. Disease assessment and yield loss. In: Cooke, B. M., Gareth Jones, D. and Kaye, B. (Eds.), The Epidemiology of Plant Diseases, 2nd edition,. Springer, pp: 43-80.

Dumalasov, V. and Bartoš, P. 2007. Reaction of spring wheat cultivars to common bunt caused by *Tilletia tritici* (Bjerk.) Wint. and *Tilletia laevis* (Kühn). Czech Journal of Genetic and Plant Breeding, 43 (3): 82-86.

El-Naimi, M., Toubia-Rahme, H. and Mamluk, O. F. 2000. Organic seed treatment as a substitute for chemical seed treatment to control common bunt of wheat. European Journal of Plant Pathology, 106: 433-437.

Entesari, M., Sharifzadeh F., Ahmadzadeh, M. and Farhangfar, M. 2013. Seed Biopriming with *Trichoderma* Species and *Pseudomonas fluorescent* on growth parameters, enzymes activity and nutritional status of soybean. International Journal of Agronomy and Plant Production, 4: 610-619.

Ershad, D. 1977. Fungi of Iran. Plant Pest and Diseases Research Institute, , no 10, 277 pp.

Fravel, D. R. 2005. Commercialization and implementation of biocontrol. Annual Review of Phytopathology, 43: 337-359.

Ghannoum, M. A. and Rice, L. B. 1999. Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clinical Microbiology Review, 12: 501-517.

Harman, G., Howell, C., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma* species-opportunistic avirulent plant symbionts. Nature Reviews of Microbiology, 2:43-56.

Hoffmann, J. A. 1982. Bunt of wheat. Plant Disease, 11: 979-986.

Jayaraj, J. Radhakrishnan, N. V. and Velazhahan, R. 2006. Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum.* Archives of Phytopathology and Plant Protection, 39 (1): 1-8

Karthikeyan, A., Parthasarathy R. and Manikandan A. 2005. Isolation of *Trichoderma Viride* and *Pseudomonas Fluorescens* organism from soil and their treatment against rice pathogens Journal of Microbiology and Biotechnology Research, 3: 77-81.

Kleifeld, O. and Chet, I. 1992. *Trichoderma harzianum* interaction with plants and effect on growth response. Plant and Soil, 144 (2): 267-272.

Kücük, C. and Kivanc, M. 2005. Effect of formulation on the viability of biocontrol agent *Trichoderma harzianum* conidia. African Journal of Biotechnology, 4: 483-486.

Kumar, S. 2013. *Trichoderma*: a biological weapon for managing plant diseases and promoting sustainability. International Journal of Agriculture Science and Medical veterinary, 1 (3): 106-121.

Lewis, J. A. and Papavizas, G. C. 1985. Characterization of alginate pellets formulated with *Trichoderma* and *Gliocladium* and their effect on the proliferation of the fungi in soil. Plant Pathology, 34: 571-577.

Liatukas, Z. and Ruzgas, V. 2009. Effect of air temperature on common bunt (*Tilletia caries*) infection in winter wheat, Acta Agriculturae Scandinavica, Section B - Soil and Plant Science, 59 (3): 225-232.

Łukanowski, A. 2006. Alternative methods of seed treatment against common bunt of wheat. Phytopatologia Polonica, 41: 79-82.

Mamluk, O. F. and Zahour, A. 1993. Differential distribution and prevalence of *Tilletia foetida* (Wallr.) Liro and *T. caries* (DC) Tul. on bread wheat and durum wheat. Journal of Phytoopathologia Mediterranea. 32 (1): 25-32.

Marfori, EC., Kajiyama, S., Fukusaki, E. and Kobayashi, A. 2003. Phytotoxicity of the tetramic acid metabolite trichosetin. Phytochemistry, 62: 715-721.

Martínez, M. A., Roldán, A. and Pascual, J. A. 2009. Performance of a *Trichoderma harzianum* bentonite-vermiculite formulation against *fusarium* wilt in seedling nursery melon plants. Horticultural Science, 7: 2025-2027.

Mastouri, F., Björkman, T. and Harman, G. E. 2010. Seed treatment with *Trichoderma* *harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology, 100 (11): 1213-1221.

Mehrabi, K. M., Zafari, D. and Sharifnabi, B. 2009. Control of Wheat Common Bunt by Mustard Flour, *Trichoderma* Isolates and Biological Materials. Journal of Science and Technology of Agriculture and Natural Resources, 47: 741-747.

Murray, GM. and Wright, DG. 2007. National contingency plan for *Tilletia controversa*, the cause of dwarf bunt of wheat. part I: pest risk assessment. plant health Australia, Canberra, Acta Australia, 16 pp.

Nayakaa, S. C., Niranjanaa, S. R. Uday A. C. Shankara, S. Niranjan Raja, M. S. Reddyb, H. S. and Mortensenc C. N. 2008. Seed biopriming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisins in maiz, Archives of Phytopathology and Plant Protection: 1-19.

Nielsen, B. J., Borgen, A. and Kristensen, L. 2000. Control of seed borne diseases in production of organic cereals. In: The Brighton Conference, pest and diseases. British Crop Protection Council, Great Britain: 171-176.

Parlak, Y. 1981. Seedborne pathogens on wheat (particularly smuts). European and Mediterranean Plant Protection Organization Bulletin, 11: 83-86.

Rao, M. S., Reddy, P. P. and Nagesh, M. 1998. Evaluation of plant based formulations on *Trichoderma harzianum* for the management of *Meloidogyne incognita* on egg plant. Nematologia Mediterranea, 26: 59-62.

Sargin, S., Yüksel, G. Y., Eltem, R. and Vardar, F. 2013. Micropropagule production from *Trichoderma harzianum* EGE-K38 using solid-state fermentation and a comparative study for drying methods. Turkish Journal of Biology, 37: 139-146.

Shaban, GM. and El-Komy, H. M. 2001. Survival and proliferation of alginate encapsulated *Trichoderma* spp. in Egyptian soil in comparison with allyl alcohol soil fumigation. Mycopathologia, 151 (3): 139-46.

Sharifnabi, B. and Hedjaroud, G. H. A. 1992. Occurrence and geographical distribution of *Tilletia* species attacking winter wheat in west and northwest of Iran. Iranian Journal of Plant Pathology, 28: 85-96.

Sharma, P., Patel, N. P., Saini, M. K. and Deep, S. 2012. Field demonstration of *Trichoderma harzianum* as a plant growth promoter in wheat (*Triticum aestivum* L). Journal of Agricultural Science, 4 (8): 65-73.

Solanki, M. K., Singh, N., Singh, R. K., Singh, P., Srivastava, A. K., Kumar, S., Kashyap, P. L. and Arora, D. K. 2012. Plant defense activation and management of tomato root rot by a chitin fortified *Trichoderma* formulation. Phytoparasitica, 39 (5): 471-481.

Subash, N., Viji, J., Sasikumar, C. and Meenakshisundaram, M. 2013. Isolation, media optimization and formulation of *Trichoderma Harizanum* from agricultural soil. Journal of Microbiology and Biotechnology Research, 3 (1): 61-64.

Taylor, A. G., Min, T. G., Harman, G. E., and Jin, X. 1991. Liquid coating formulation for the application of biological seed treatments of *Trichoderma harzianum*, Biological Control 1 (1): 16-22.

Vanden Bossche, H., Marichal, P., Gorrens, J. and Coene, M. C. 1990. Biochemical basis for the activity and selectivity of oral antifungal drugs. British Journal of Clinical Practice. 71: 41-46.

Verma, M., Brar SK. and Tyagi, RD. 2007. Antagonistic fungi, *Trichoderma* spp. Panoply of biological control. Biochemical Engineering Journal, 37: 1-20.

Vinale, F., Flematti, G., Sivasithamparam, K., Lorito, M., Marra, R., Skelton, BW. and Ghisalberti, EL. 2009. Harzianic acid, an antifungal and plant growth promoting metabolite from *Trichoderma harzianum*. Journal of Natural Products, 72: 2032-2035.

Warrior, P. 2002. Formulation of biological control agents for pest and disease management. In: Warrior, P., Konduru, K.

and Vasudevan, P. (Eds.), Biological Control of Crop Diseases. Marcel Dekker, New York, pp: 421-442.

Wilcoxson, R. D. and Saari, E. E. 1996. Bunt and Smut diseases of wheat: concepts and methods of disease management. Mexico, CIMMYT, 66 pp.

Yüksel, H., Güncan, A. and Doken, M. T. 1980. The distribution and damage of bunts (*Tilletia* spp.) and wheat gall nematode [*Anguina tritici* (Steinbuch) Chitwood] on wheat in the eastern part of Anatolia. Journal of Turkish Phytopathology, 9: 77-88.

Zscheile, F. P. 1955. Some physiological aspects of bunt resistance in wheat. Plant Physiology, 30: 432-437.

# بررسی اثر حالت فیزیکی فرمولاسیون بر توانایی *Trichoderma harzianum 199* در کنترل سیاهک پنهان گندم

**سیدمهدی شتاب بوشهری**

بخش تحقیقات گیاه‌پزشکی مرکز تحقیقات کشاورزی و منابع طبیعی خوزستان، ایران.
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**چکیده**: به‌منظور بررسی امکان جایگزینی قارچ‌کش‌های شیمیایی با مواد محافظت کننده بیولوژیکی سه فرمولاسیون به شکل‌های متفاوت فیزیکی (مایع، پودر و سوسپانسیون مخلوط ژل) از *Trichoderma* 199 *harzianum* در سطح آزمایشگاهی تولید و برای کنترل سیاهک پنهان گندم در اثر قارچ *Tilletia laevis* Kühn. در رقم چمران در مقایسه با قارچ‌کش تتراکونازول به روش تیمار بذر در یک آزمایش مزرعه‌ای مورد بررسی قرار گرفت. نتایج حاصل از تجزیه و تحلیل داده‌های آزمایش نشان داد که بین تیمارهای آزمایش تفاوت معنی‌دار وجود دارد). پس از تتراکونازول (۹۷/۹٪ کاهش بیماری)، کمترین میزان بیماری در تیمار فرمولاسیون سوسپانسیون مخلوط ژل (۴۳/۴٪ کاهش بیماری) و بیشترین میزان سیاهک در تیمار فرمولاسیون مایع مشاهده گردید (۲۵/۳۱٪ افزایش بیماری). فرمولاسیون پودر تالک رویداد بیماری را (۳۹/۰۷٪) کاهش داد. تجزیه واریانس اثرات تیمارها بر میزان عملکرد دانه و اجزا مهم عملکرد محصول نشان داد که تیمار سوسپانسیون مخلوط ژل علیرغم کنترل بهتر بیماری بعد از دو تیمار دیگر شامل پودر تالک و فرمولاسیون مایع قرار گرفت. نتایج حاصل از این آزمایش نشان می‌دهد که *Trichoderma* 199 *harzianum* توان کاهش سیاهک پنهان گندم را داراست، اما نوع فرمولاسیون مورد استفاده آن تأثیر معنی‌داری بر میزان کنترل بیماری و اجزای عملکرد گندم دارد.

**واژگان کلیدی**: تیمار بیولوژیک بذر، فرمولاسیون، قارچکش بیولوژیک، سیاهک پنهان گندم، گندم ارگانیک