RESEARCH PAPER

Improvement of Resistance of Forage Beans to Pathogens by Compositing Them with SiO_2 and Chitosan Nanoparticles

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ABSTRACT

Nanotechnology can offer advantages to pesticides, like reducing toxicity, improving the shelf-life, and increasing the solubility of poorly water-soluble pesticides, all of which could have positive environmental impacts. Vicia faba L. is a high-protein forage and vegetable crop with a high productivity potential limited by pathogenic mycoflora. Fusariomycoses are dominant in the bean mycoses complex. Fusariosis are widely known phytomycoses globally with a wide range of mycotoxins, and beans are used for food, including in dietetics, and livestock feed, all over the world. This work aimed to select vegetable and forage beans with different degrees of resistance to Fusarium seedlings. For several years (1999-2020) in the soil and climatic conditions of the city of Belgorod (south of the central black earth region of Russia) on the territory of the National Research University "BelGU", a collection of beans for fodder and vegetable use was studied against a natural infectious background. Fusariosis appeared annually at all stages of the growing season of broad beans. Severe damage to the seeds led to the death of seedlings. In some years, the prevalence of fusarium disease reached 72%, and the loss of grain yield - 68%. Fusarium sporotrichioides and Fusarium oxysporum were isolated and identified from the affected seedlings. Moreover, the former predominated on seedlings, the latter - on adult plants. Most of the tested varieties of beans (69%) were characterized by low resistance. No immune and fusarium-resistant bean varieties have been identified.

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INTRODUCTION

Nanotechnology has led to the development of new concepts and agricultural products with immense potential to manage the aforementioned problems. Nanotechnology has substantially advanced in medicine and pharmacology, but has received comparatively less interest for agricultural applications [1,2]. The use of nanotechnology in agriculture is currently being explored in plant hormone delivery, seed germination, water management, transfer of target genes, nanobarcoding, nanosensors, and controlled release of agrichemicals [3]. Material scientists have engineered nanoparticles with

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desired characteristics, like shape, pore size, and surface properties, so that they can then be used as protectants or for precise and targeted delivery via adsorption, encapsulation, and/or conjugation of an active, such as a pesticide [4]. As agricultural nanotechnology develops, the potential to provide a new generation of pesticides and other actives for plant disease management will greatly increase. Broad beans (*Vicia faba* L.) is a cultivated plant from the Legume family, which is currently not found in the wild. Beans are a high-protein forage and vegetable crop, an excellent precursor and honey plant. The productivity potential of beans is high, but it is largely limited by pathogenic mycoflora [5]. Fusarium plant infection is one of the most common and harmful in the world [6-11]. Diseases of fusarium etiology are also dominant in the complex of mycoses of fodder and vegetable beans [12,13].

A complex of Fusarium fungi participates in the defeat of plants, many of which are ecologically plastic and widespread in all regions of Russia [14]. Mycotoxins *Fusarium* belong to the priority contaminants of food raw materials and food products that pose a danger to humans and animals [11-19]. Fungi of the genus *Fusarium* are opportunistic, or potentially pathogenic for humans and animals [20-22], can cause necrosis and ulcers on nails, fingers [23]. There is evidence that fusariotoxins are carcinogens (cause cancer of the esophagus), can cause toxicosis, aleukia and gastrointestinal diseases in humans [24-26]. These substances cause the development of equine leukoencephalomalacia, pulmonary edema in pigs, hepatosis and dyschondroplasia in chickens, and "deterioration in egg quality" syndrome in chickens [27-31]. Fusariums are widely known phytomycoses in the world with a wide range of mycotoxins, and beans are used for food, including in dietetics, and for livestock feed, all over the world. At the moment, there are no varieties of beans completely resistant to fusarium. Therefore, the problem of resistance of forage beans to Fusarium pathogens is urgent. The purpose of this work was to select varieties of vegetable and forage beans with different degrees of resistance to fusarium seedlings.

MATERIALS AND METHODS

For a number of years (1999-2020), in the soil and climatic conditions of the city of Belgorod (south of the central chernozem region of Russia) on the territory of the National Research University «BelGU», a collection of beans (200 varieties) for fodder and vegetable use was studied against a natural infectious backgroundSowing of the faba bean's varieties and care were carried out manually in accordance with the method of B.A. Dospekhov (1979) and the requirements of zonal agricultural technology without the use of fertilizers and pesticides. A wide-row sowing method was used, with a seeding rate of 0.3 million / ha. Crop care included post-sowing crust control, inter-row cultivation as the crops became clogged and after rains. The area of the accounting plot was 2 m2 with 2 replicates. Determination of plant diseases was carried out using keys Shkalikova et al. (2010), Gritsenko et al. (2008) and atlas by Booth (1971) and Gerlach (1982).

The prevalence of the disease was calculated using the formula: $P = (100×n) / N$, where n – the number of infected plants in which at least one organ had a score of 1 or more, N – total number of plants in the sample, 100 – conversion of an indicator to a percentage. The shortfall, or losses, of the crop, was expressed as a percentage and was determined by the formula: $Q = (A–a)×100/A$, where A – harvest healthy plants, a – harvest of diseased plants (Shcherbakova, 2013). Isolated leaves of bean plants of 16 varieties were inoculated in the laboratory of mycology of the Department of Biotechnology and Microbiology of the National Research University "BelGU" with a suspension (5 ml) of pathogen spores in sterile water ($1x10^6$ conidia / ml). The leaves were incubated at a temperature of + 23 °C in a humid chamber. On the 4th day, the symptoms of the disease were described. In the control, the leaves were sprayed with water.

Bean leaf width varied by cultivars, therefore the size of spots after inoculation was evaluated in points according to a 4-point 5-step international scale, according to which 0 points (immune cultivars) are assigned when the leaf area is affected up to 10%, 1 point (resistant) - when 11-25%, 2 points (medium resistance) - at 26-50%, 3 points (weak resistance) - at 51-75% and 4 points (unstable varieties) - if 76-100% of the leaf is affected.

RESULTS AND DISCUSSION

The use of nanoparticles to protect plants can occur via two different mechanisms: (a) nanoparticles themselves providing crop protection, or (b) nanoparticles as carriers for existing pesticides or other actives, such as double-stranded RNA (dsRNA), and can be applied by spray application or drenching/soaking onto seeds, foliar tissue, or roots. Nanoparticles, as carriers, can provide several benefits, like (i) enhanced shelf-life, (ii) improved solubility of poorly water-soluble pesticides, (iii) reduced toxicity, and (iv) boosting site-specific uptake into the target pest. Another possible nanocarrier benefit includes an increase in the efficacy of the activity and stability of the nanopesticides under environmental pressures (UV and rain), significantly reducing the number of applications, thereby decreasing toxicity and reducing their costs (Fig. 1).

The use of chitosan nanoparticles to manage rice blast disease pathogen, our findings showed that chitosan nanoparticles at a concentration of 350 ppb have also shown strong antifungal activity against the *Pyricularia oryzae* fungus, as presented in Fig. 2.

Chitosan nanocomposite-based chitosan hydrogels (Chit/NCs hydrogel) have been prepared using metal vapor synthesis (MVS). Also, SEM measurements revealed damage to *A. flavus* cell membranes. Current findings indicate that the

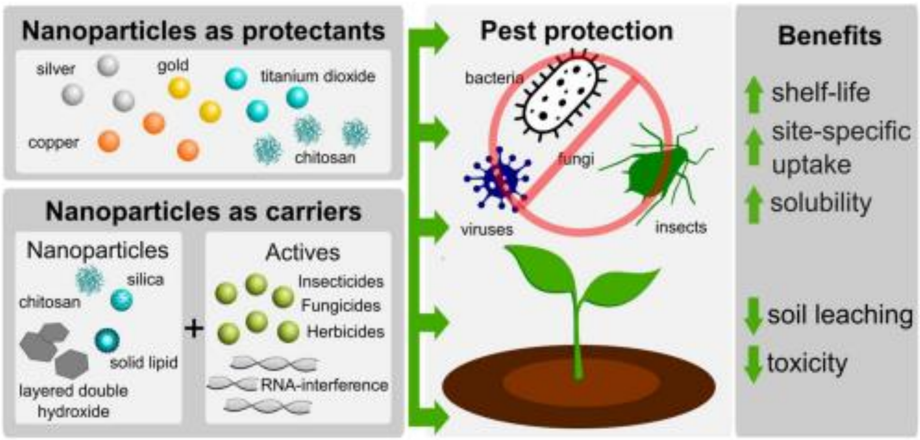

Fig. 1. Nanomaterials as protectants or carriers to provide crop protection. This schematic shows different nanomaterials as either protectants or carriers for actives such as insecticides, fungicides, herbicides, or RNA-interference molecules, targeting a wide range of pests and pathogens. It also highlights the potential benefits of nanomaterial applications, such as improved shelf-life, target site-specific uptake, and increased solubility, while decreasing soil leaching and toxicity.

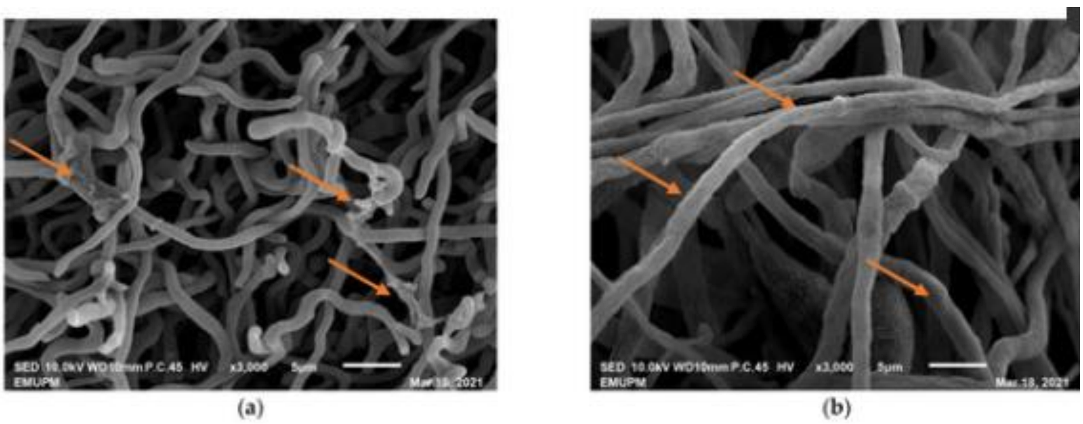

Fig. 2. Micrographs of the scanning electron microscope (SEM) at a magnification of X3000. (a) Mycelia of *Pyricularia oryzae* **fungus treated with chitosan nanoparticle, the mycelial growth was smaller with breakage at some points when compared to the control. (b) For the control, the mycelia of** *Pyricularia oryzae* **grown bigger, thicken, and without any breakage.**

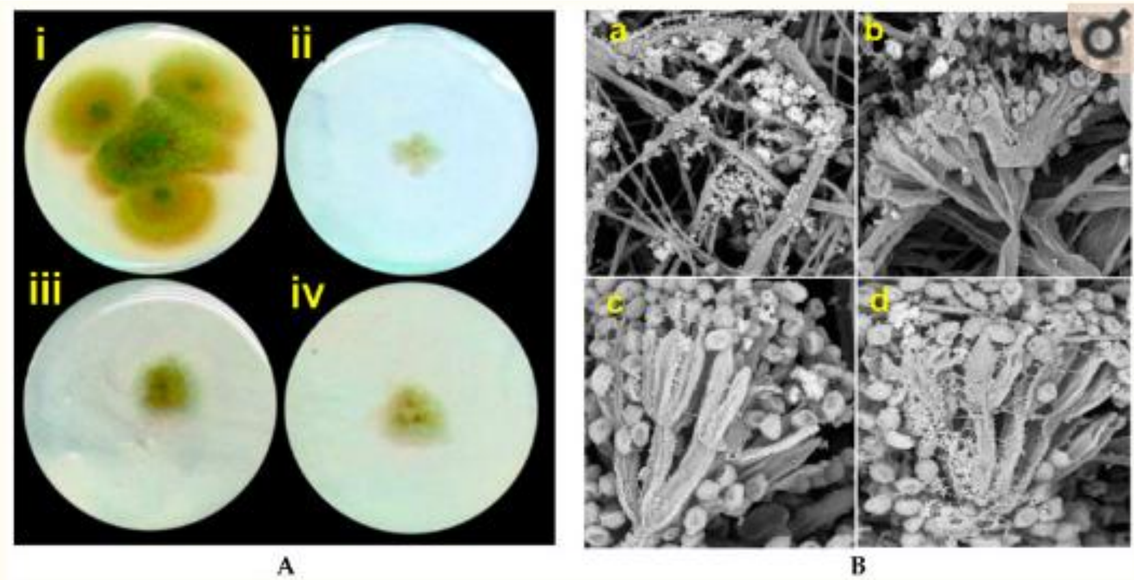

Fig. 3. (A) Antifungal activity of Chit NCs against *A. flavus* collected from feed samples. (i), control (without nanocomposite treatment), (ii), (iii), and (iv) fungal mat treated with 30, 60, and 90 milligrams of nanocomposites. All petri dishes treatment was incubated at 28 °C for 10 days. (B) Fungal mycelium of *P. expansum* treated with Chit NCs referred to the morphological changes in fungal hyphae, SEM images depicted markedly shriveled, crinkled cell walls, and flattened hyphae of the fungi (a), hyphal cell wall and vesicle damaged (b), irregular branching (a and b), and collapsed cell, formation of a layer of extruded material (d) Source (Abd-Elsalam KA. unpublished data).

Fig. 4. Fusariosis seedlings of vegetable beans.

antifungal activity of nanocomposites in vitro can be beneficial depending on the type of fungal strain and the concentration of nanocomposites (Fig. 3A). Chit/NCS hydrogel is a revolutionary nanobiopesticide developed by MVS used in food and feed to induce plant protection against mycotoxigenic fungi [32]. The fungicidal behavior of chitosan-silver nanocomposites (Chit-NCs) against *Penicillium expansum* from the feed samples was investigated. Chit-NCs < 10 nm in size have an important antifungal inhibitory effect against *P. expansum*, the causative agent of blue mold-contaminated dairy cattle feed [33]. *P. expansum* treated with Chit *P. expansum* treated with Chit NCs was investigated by HR-SEM, alterations in conidiophores, metulae, phialides, and mature conidia characteristics had been observed to obtain information about the mode of action of Chit-NCs (Fig. 3B). Therefore, nanocomposites can be utilized as viable alternatives to the already available arsenal of fungicides.

Fusariosis appeared annually at all stages of the growing season of beans. Affected seedlings quickly turned yellow (see Fig. 4), wither and die. Darkened vessels were observed on transverse sections of stems and roots (see Fig. 4).

Severe damage to the seeds led to the death of seedlings. In some years, the prevalence of fusarium disease reached 72%, and the loss of grain yield - 68%. From the affected seedlings were isolated and identified *Fusarium sporotrichioides* Sherbakoff (section *Sporotrichiella*) and *Fusarium oxysporum* Schlechtendahl (section *Elegans*). Moreover, the former prevailed on seedlings, the latter - on adult plants. It should be noted that fusarium wilting was accompanied by the colonization of weakened forage beans with *Alternaria*, and is consistent with the data of other authors, who noted the charred species of plants. According to [34,35] species *F. oxysporum* are relatively weak pathogens, while the species *F. sporotrichioides* causes widespread latent infection of grain [36,37]. Therefore, inoculation of leaves in the laboratory was carried out with a spore suspension *F. sporotrichioides*.

Most of the tested varieties of beans (69%) were characterized by low resistance (3 points). Of all varieties only 25% (for example Russian Black, Alfred, Pirkkonen, Geo) are low-medium resistence (2.5 points) (see Fig. 5). And varieties like Ar-ban-cin-hu-dou (China), Josny (Poland),

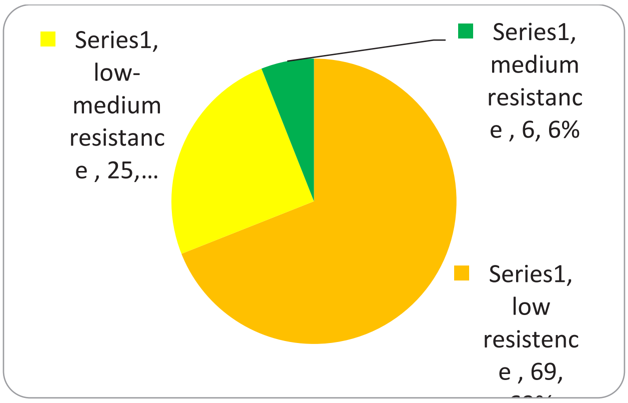

Fig. 5. Development of the disease in different varieties of *Vicia faba* L.

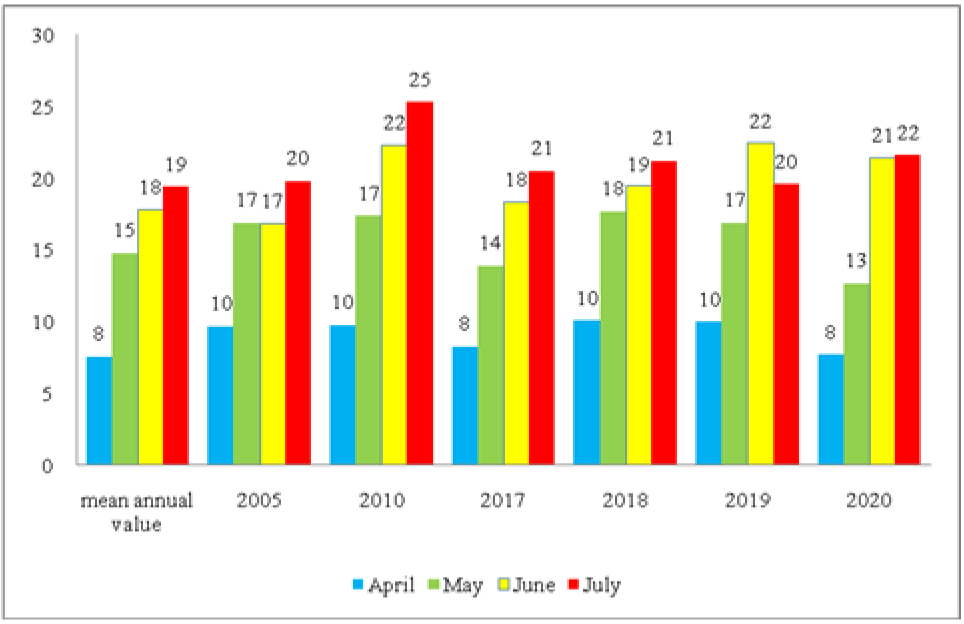

Fig. 6. Temperature (°C) during the growing season of *Vicia faba* L.

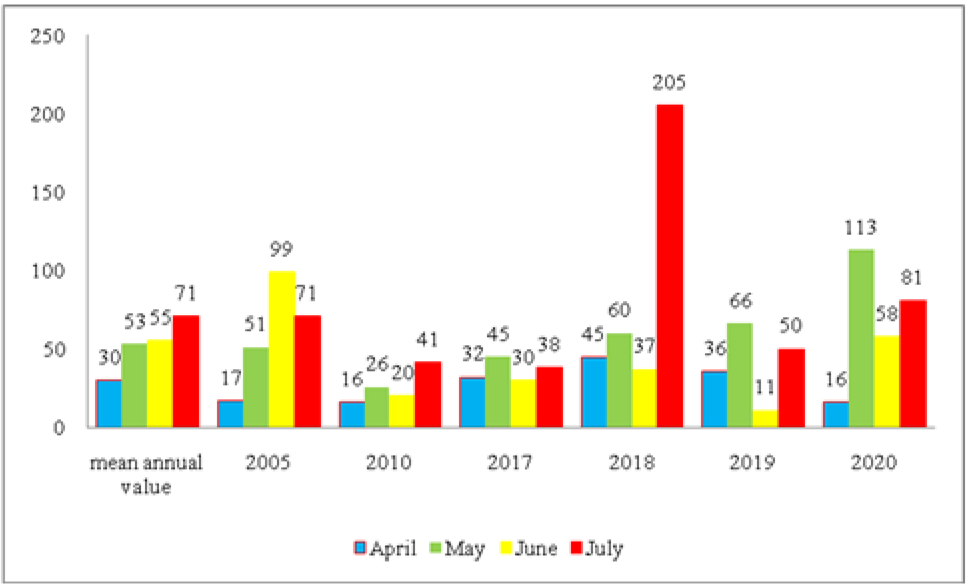

Fig. 7. Amount of precipitation (mm) during the growing season of *Vicia faba* L.

Chlumesky, Roschutjer Feldbohn и Stofil (Czech Republic) are medium resistance (2 points). No immune and fusarium-resistant bean varieties were identified (0-1 points).

Varieties with rapid growth at the initial stages of development may be of interest to breeders, due to which, in natural conditions, they will have a chance to “escape” from mass infection of seedlings with Fusarium. In our study, these are varieties Russian Black, Aquadul, Soving.

It is known that the main factor in the spread of fusarium is the soil, and the additional factor is seed, air currents, and raindrops (Chulkina et al. 2008). The manifestation and development of fusariosis is also facilitated by the violation of the correct alternation of crops. Studies have shown that the weather conditions in May-June play an important role in the manifestation of *Fusarium*. So, for example, May in 2005 and 2019 was humid and warm (excess over the average annual values for temperature and precipitation in Figures 6 and 7), when diseased plants lost their turgor, turned yellow, turned black and shriveled. Such weather contributed to the spread of root rot on the crops of forage beans, when the white cobweb mycelium of the fungus was clearly visible in the area of the root collar. May and June 2017 were cool and dry (see Fig. 6 and Fig. 7), which favored fusariosis on the seedlings, which turned yellow and easily pulled out of the soil.

To protect plants from fusariosis, it is necessary to combine agrotechnical measures with the use of pesticides and the correct selection of varieties. For example, using a wide-row sowing method creates favorable conditions for the growth of beans, which increases the natural resistance of plants. Varieties with rapid growth at the initial stages of development may also be of particular interest to breeders, due to which, under natural conditions, they will have a chance to “escape” from mass infection of seedlings with Fusarium, for example, varieties Russian Black (Russia), Aquadul (Holland), Soving (Sweden), Survoy (France), Britz (Canada), Sinabe-Im (Peru).

CONCLUSION

Our findings have indicated that the control of toxigenic fungi and the detoxification of mycotoxins are not adequate for sustainable agricultural ergonomics. Therefore, novel treatment methods for improving the food safety and protection must be applied. Nanohybrid antifungals are thus, of primary importance for a synergistic approach to resolve diverse problems in the management of fungal pathogens causing agricultural/post-harvest diseases in the 21st century, with a focus on Green Nanotechnology, which is environmentally sustainable and provides a continuum for the plant, animal and human health. The nano-hybrid anti-fungals are anticipated to cater to the need of the growers, consumers as well as the environment activists through rapid, effective, and comparatively improved eco-safety attributes for controlling the yield and produce quality deterring potential of the fungal phytopathogens. Fusariosis appeared at all stages of the growing season of *Vicia faba*. The prevalence of fusarium disease reached 72%, and the loss of grain yield - 68%. *Fusarium sporotrichioides* (predominated on seedlings) and *Fusarium oxysporum* (on adult plants) were isolated and identified from the affected seedlings. And 69% of the tested varieties of *Vicia faba* were characterized by low resistance. The varieties Ar-ban-cin-hu-dou, Josny, Chlumesky, Roschutjer Feldbohn и Stofil are medium resistance and may be of interest for breeding for resistance to fusariosis in seedlings of broad beans. Also promising for select the varieties, as Akvadul, Russian Black, Soving, Survoy, Britz, Sinabe-Im, whose plants grow rapidly at the initial stages of development, so they have a chance to “escape” from the mass infection of seedlings with fusariomycosis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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