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Improvement in Engineering Design of Machines for Biological Crop Treatment with Microbial Products

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Abstract: Evidence of the need to use sustainable farming practices oriented towards wide application of microbial products is provided in the study based on the data of the negative effect modern technology has due to anthropogenic environmental impacts. It is established that traditional crop treatment practices using the existing machines decrease biological product effectiveness significantly as compared to laboratory data. A number of factors were studied that can influence microbial viability in biological products used to treat seeds and vegetative plants with the help of the chemical crop protection machines. It was revealed that the processes characterized by cavitation have the greatest negative impact. The specified factors are studied through comparison of conventional and sustainable farming technologies. Recommendations were elaborated for development and improvement of machines used to treat crops with microbial products.

Key words: Sustainable farming, machines for chemical crop protection, biological products, microorganisms, viability, improvement,

INTRODUCTION

The world wide use of chemical crop protection practices in arable farming led to increased amounts of synthetic pesticides exceeding 6 million tons per year in the early 2000s and caused a number of global problems (Carvalho, 2017). The most urgent problems among them are natural soil fertility fall, agricultural land degradation and loss (Lal, 2015), aggravated by changes in the agrochemical properties of soil due to the application of mineral fertilizers, spread of drug-resistant pathogens and harmful insects (Hobbelen *et al*., 2014; Lucas *et al*., 2015), product quality deterioration and in consequence lower economic indices in the agricultural sector. To address the negative effects, transition to sustainable farming development is proposed which implies a focus shift and increased attention to agrobiological factors, taken into account both in assessment of the technology in general and of operations in particular. The diagram of the purposes of the main crop cultivation operations (Fig. 1) demonstrates both conventional agrotechnical and biological factors. The proposed approach enables the transition from intensive to sustainable practices to be comprehensively assessed and the potential for further machine improvement to be considered taking into account not only agrophysical but also microbiological indices (Fig. 1).

The long-lasting studies show that most of the life processes in plants take place when interacting with microflora (Ho *et al*., 2017), the plant being considered as an “organizer” of the whole microbial community (Garipova *et al*., 2017). However, despite the microbial diversity and numerous reports on successful application of microorganisms used to increase crop productivity and resistance, the share of microbiological products used in crop production remains insignificant. The study highlights some of the most relevant factors retarding their wide use.

Although, the effects of tillage and chemicals use on soil biota have been studied to some extent (Geisseler and Scow, 2014; Smith *et al*., 2016), too little attention is paid to the effects of technological strains microorganisms suffer from in operations involved in crop treatment with biological products. For example, to ensure effective use of the broad spectrum microbial product Nitrofix (composed of *Bradyrhizobium jiaponicum* and *Bradyrhizobium elkanii* bacteria immobilized on the Bio-clean peat) it is recommended the seeds treated with microbial inoculants be sown within 24 h. The greatest

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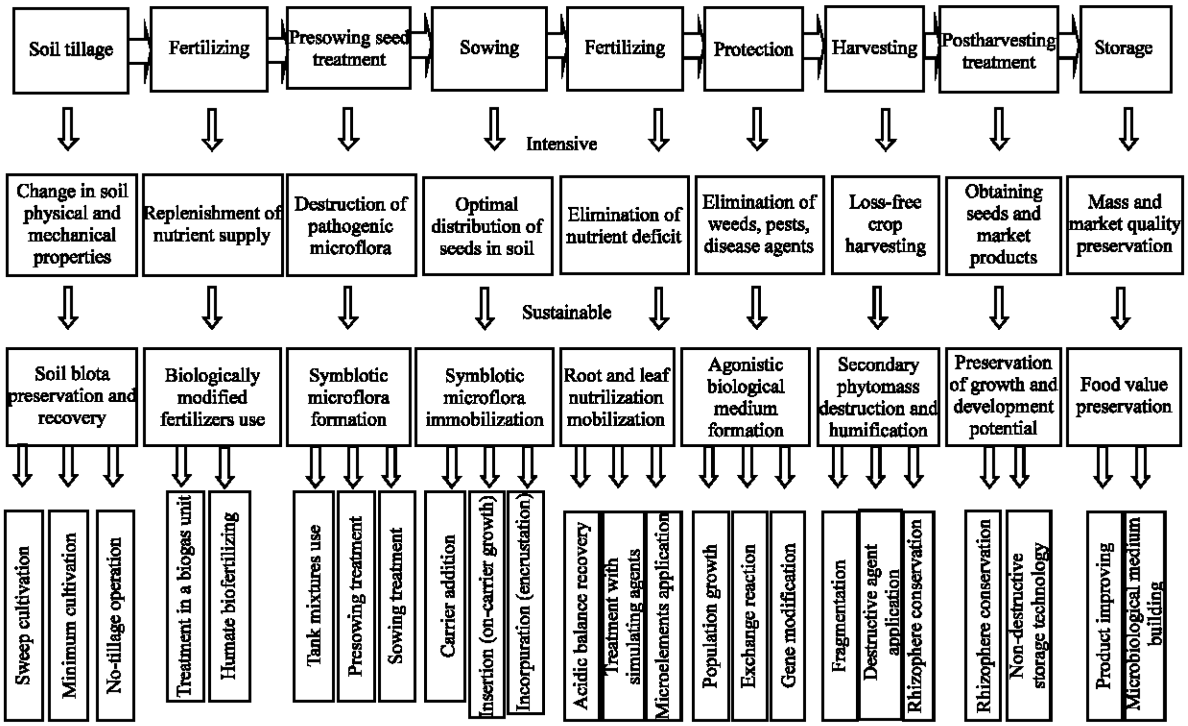

Fig. 1: Diagram of intensive and sustainable technologies purposes

effect is achieved when the seeds are treated no more than 2 h before sowing with direct sunlight being avoided. Similar requirements are specified on the use of the widespread “Rizotorfin” bioproduct. Along with this, most microorganisms in biological products used in crop production have a narrow storage temperature range, a limited working fluid application time period and a number of other parameters. The physical and mechanical impact of crop protection machine operation on the viability of useful microflora has not been studied.

The research, therefore, aims to examine the effect of crop protection machine operation on the viability of *Bacillus subtilis* bacteria including those contained in commercial biological products. Biological products were assessed in terms of their response to excessive static pressure and transporting through pressure lines equipped with fittings, gear-type pump operation, mechanical mixing and mist formation by means of mechanical and hydraulic atomizers.

MATERIALS AND METHODS

Four strains of *Bacillus subtilis* Cohn bacteria: 24D, 11VM (selected at ARRIAM, All-Russia Research Institute for Agricultural Microbiology) No.519) and the strains of *B. subtilis* 1 and 2 isolated and identified in the Biotechnology Laboratory of Bashkir State Agrarian University (Federal State Budgetary Educational Institution of Higher Education) were used in the tests. Bacteria were grown in a liquid medium composed of (g/L), meat infusion (0.5 kg of meat per 1 L of water broth), peptone (Panreac, Spain) at 10, NaCl at 5. To estimate the number of cells (titre) we used the 10 fold dilution of 0.15 M Sodium Chloride in sterile solution with 0.01M Sodium Phosphate buffer, pH 7.0 and bacteria were grown in a solid growth medium composed of (g/L), Meat-Peptone Broth (MPB) containing meat infusion (0.5 kg of meat per 1 L of water), peptone (Panreac, Spain) at 10, NaCl at 5, microbiological agar (Panreac, Spain) at 15 (MPA), at a temperature of 37°C.

To determine the response of microorganisms to excessive static pressure and hydraulic spray nozzles we used a plant made up of a sealed reservoir, a pump, a pressure gage, a pipe and a spray nozzle. The tested product of the initial concentration (~$3.5 \cdot 10^9$ CFU/mL) was exposed to excess pressure of 100, 200, 300 and 400 kPa for 60 min in turn. A test plant, a closed circuit arrangement of a reservoir, a pump and a pipeline with fittings was used to determine the impact of transporting the fluid through pressure lines with fittings and gear-type pump operation on the tested product. After passing through the circuit the product flew back into the reservoir. The minimum length of the pipe was set to assess the impact of the gear-type pump

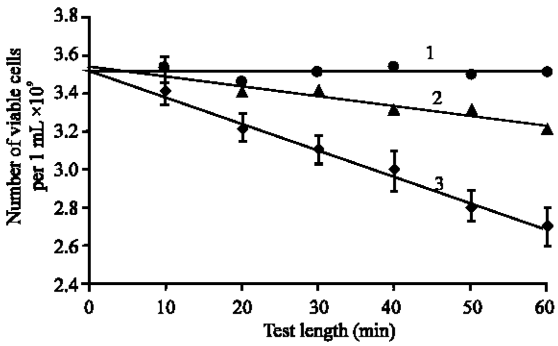

Fig. 2: The impact of the factors under study on viability of *Bacillus subtilis* 26D: 1)-Control product; 2)-Under the pump operation and 3)-Under conditions of the working fluid flow through pipelines with fittings

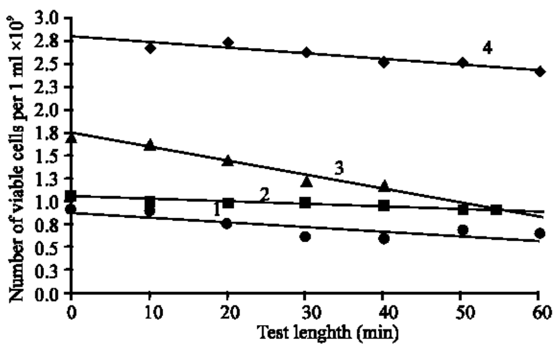

Fig. 3: The effect of mechanical action length on the number of viable cells of *B. subtilis*: 1)-Strain 1; 2)-Strain 2; 3)-Strain 26D and 4)-Strain11VM

operation, a five meter long pipe with bendings, valves and contractions was installed to assess the impact of transporting the product through pressure pipelines equipped with fittings. A plant consisting of a disk atomizer (R = 80 mm) driven by an electric motor (n = 2850 min-1) and placed in a sealed reservoir was employed to test the response of microorganisms to mechanical action (mixing, using a disk atomizer).

To assess the effect of both physical, mechanical and chemical impacts on microbial viability, samples were taken every 10 min of plants operation, the number of viable cells in Meat Peptone Agar was identified in Petri dishes (incubated at 37°C for 24 h). The value was compared to the values of the control product, the biological product not exposed to the tests and placed in the same incubation medium with the tested sample.

The number of cells in one sample was assessed in three independent retrials (Petri dishes). Figure 2 and 3 demonstrate the average values and their standard deviations.

RESULTS AND DISCUSSION

The graphs in Fig. 2 demonstrate that transporting of the tested product through a pressure pipeline with fittings and pump operation bring about a decrease in the number of bacteria capable of reproduction.

Thus, constant mixing, pump blade operation, pressure changes in the pipe fittings have a significant negative effect on microbial viability leading to destruction of a large proportion of microorganisms in cases of prolonged mechanical mixing of the working fluid.

It was also revealed that the bacteria survival depends not only on the mechanical action length and intensity but also on the microbial genotype. Figure 3 demonstrates that 11VM strain bacteria had the greatest resistance to mechanical action, even at the initial high cell concentration and 24D strain bacteria showed the weakest resistance as the number of viable cells fell by more than half within 1 h of mixing.

Along with physical and mechanical effects composition of the working fluid has a significant effect on microbial viability. Microorganisms were added to the working fluid (1 L) containing 2% of the sodium carboxymethyl cellulose glue and 3% of the dry potassium and magnesium fertilizer and circulated for 3 min at excess pressure of 1.5 bar in the test plant made up of a reservoir, a pump, fittings and a slit atomizer with a performance capacity of 2 L/min. It was revealed that under the above conditions bacteria on the Meat Peptone Agar lost their viability completely in comparison to the control product unexposed to the tests as they featured the same number of cells in the working fluid. Thus, aggressive liquid media or a change in the medium properties for example, adding an emulsifier, may enhance an external action impact significantly, leading to the complete destruction of microbial cells in the biological products.

External actions arising in the conventional mechanical crop treatment practices can significantly reduce the effectiveness of biological products when passing from laboratory and field experiments on small plots to their use in large areas under production conditions. This is accounted for by the fact that the existing designs of machines for crop treatment on large areas employ a prolonged mechanical or hydraulic mixing of the working fluid and the machines possess local resistance features which inevitably leads to cavitation (Smorodov *et al*., 2008). Cavitation, formation of bubbles (voids) in the suction zones and their subsequent implosion generating high pressures is the main factor to reduce microbial viability. This is fully in line with the conclusions of the study on the effect of physical and mechanical action on biological products, that underline significant distress and destruction of microorganisms against cavitation (Antusheva *et al*., 2014; Yusof *et al*., 2016). Based on the data obtained, we believe it is necessary to consider negative effects of the operational impacts at the stage of developing machines for treatment of crops with microbial products.

First of all, it is necessary to limit the effect of centrifugal pumps and mechanical or hydraulic mixing units which are the main elements to cause cavitation. To this end, it is recommended that the pressure of the working fluid in the system be provided by creating static pressure which implies forcing air into the reservoir or installing pumps featuring low operating member speed, such as membrane pumps. It is also recommended the length of pipelines and the number of fittings that produce local resistance be minimized.

Account should be taken of manyfold increase in the negative effect of physical and mechanical action on biological products added to multi component working fluids during the operational process as well as of strain resistance to the negative impacts in the strain selection process.

Practical use: The results report that the use of the conventional machine designs reduces the amount of live microorganisms in the working fluid. This is accounted for by the fact that the machine operational process is based on the principle in which pressure is produced and working fluid is distributed disregarding cavitation hazards.

Currently, the "Pre-Mix" technology (Fig. 4) which implies premixing of the concentrated biological product and pure water immediately before being fed to the atomizers is considered as one of the most promising for improving crop protection implements.

In order to prevent sediment the chemical product is constantly mixed at an intensive rate in a concentrate preparation mixing reservoir featuring its own pump. Despite the clear and positive potential of the technology, the use of a biological product in the system individually or combined with chemical substances does not only fail to eliminate but also intensifies the negative effect of physical and mechanical action on the microbial living cells for the reasons revealed above.

Taking the aspect into consideration and in order to address the contradiction we suggest an additional unit be installed in the water or working fluid feed line of the conventional design atomizer. The unit consists of a proportioning metering pump Mix Rite which takes in the product in the ratio from 0.2-10% of the fluid flowing through the pump and a reservoir for the biological product, installed in an insulated container which

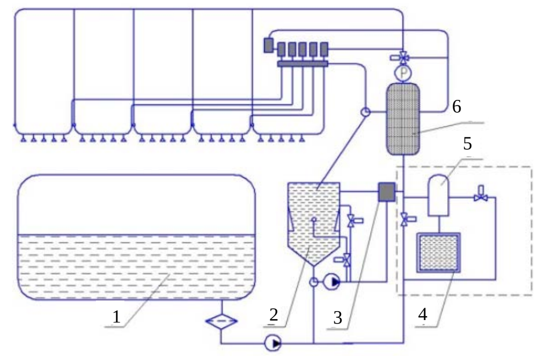

Fig. 4: The diagram of the spraying unit featuring preparatory concentrate procedures and a unit for biological product supply: 1)-Eservoir for pure water; 2)-Reservoir for concentrate preparation; 3)-Dispenser; 4)-Insulated container for the biological product; 5)-Proportioning metering pump Mix Rite and 6)-Mixing unit

maintains the optimum temperature for microorganisms, for example, at+5°C for *Bacillus subtilis* 26D (Bellow *et al*., 2007). There is no need to install additional components of the pump drive and metering is carried out through the fluid flow in the main line. Field studies, where microorganisms were exposed to negative action for a limited time, demonstrated a rise in the effectiveness of microbial products and a yield increase.

CONCLUSION

It has been established that physical and mechanical effects arising in operation of machines for treating crops with protective and growth stimulating products, reduce microbial viability and in some cases lead to destruction of the cells.

RECOMMENDATIONS

With this in view, the following recommendations have been made.

- To reduce the time of exposure or completely eliminate the effect of mixing units causing cavitation on the working fluid containing microorganisms
- To provide pressure of working fluid by creating static pressure which implies forcing air into the reservoir or installing pumps featuring low operating member speed such as membrane pumps
- To minimize the length of pipelines and limit the number of fittings that create local resistance

Manyfold increase in the negative effect of physical and mechanical action on biological products is observed in multi component working fluids containing chemical substances.

Different strains of even one type of microorganisms possess various resistance to external physical and mechanical impacts which should certainly be taken into account in the strain selection process.

It must be reported that insufficient attention is currently being paid to the evaluation of what impact the operational processes of farm machinery of machines and mechanical implements for treating crops with biological products in particular have on microbial viability. Considerations made on the negative impact of external action on microbial viability may help to avoid mistakes in design and engineering solutions related to the development of machinery for treating crops with biological protection and growth stimulators and the reported scheme for the supply of biological products ensures microbial viability, their better activity and effectiveness.

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