

Vitrofur

VITROFURAL® is presented as a microbe contamination inhibitor in cultivation media for the massive production of vitro-plants, replacing the conventional process of autoclave sterilization. It is bottled in amber-colored 6 g. vials, with screwed on plastic lids, and in cardboard box secondary containers holding 10 vials. The recommended concentration for VITROFURAL® in the cultivation medium is 116 mg/L, therefore with 6 grams, 50 L of cultivation medium can be prepared. The expiration date is 5 years from the date of manufacture, keeping it away from the light and damp. The product is accompanied by a declaration of conformity with its quality specifications, correctly elaborated and implanted according to Cuban norm NC 1090:2015.

VITROFURAL® was registered in Cuba with permit number 013/99 in 1999, where its use has become extensive in all bio-factories belonging to the company producing and commercializing seeds for the Ministry of Agriculture, as well as in agricultural biotechnology centers such as the Centro de Bioplasmas and the Instituto de Biotecnología de las Plantas (IBP), with excellent results in the micro-propagation of potatoes, bananas, papaya, plantains, sugar cane, pineapple, taro and forestry products, etc. Its efficacy has been verified in 29 Latin American laboratories in cultivation media for potatoes, plantains, garlic, citrus fruits, cherries, cranberries, chrysanthemums and sugar cane. Currently the product is being used in institutions in Mexico, Chile, Peru, Ecuador and Brazil, an evident demonstration of the advantages represented by its use, as compared to the traditional method of autoclave sterilization.

The success of biotechnological plant propagation systems depends to a large extent on the control and prevention of contamination by microbes, something that is still one of the principal and most severe problems for plant micropropagators in the world. In the struggle to prevent or eliminate contamination by microbes in the in vitro cultivation of plants, different alternatives have been tried and put into practice. These go from increasing aseptic measures and the treatment of donor plants, to subcultures of seedlings in the cultivation medium with synthetic ornatural anti-microbe products.

Research developed by the Chemical Bioactives Center of the Marta Abreu Central University of Las Villas(Cuba) had the result of obtaining a formula going by the commercial name of VITROFURAL®, a solid dispersion containing 1-(5-bromo-fur-2-il)-2-bromo-2-nitroetene (Furvina) as the active pharmaceutical ingredient to 30 %; its first characteristic is that it has a broad range bactericidal and fungicidal effects, proven in the principal contaminant that presented in in vitro cultivation, in concentrations not exceeding 35 mg/L of the active pharmaceutical ingredient. The other great advantage of this product, the one that has been the most important limiting element for the use of other chemical sterilizers, is that it presents nophytotoxic effects on the concentration controlling contamination.

The Plant Biotechnology Institute (Villa Clara, Cuba) is the referential bio-factory and it offers courses and training sessions that include training in the use of VITROFURAL® as one of the ways to increase efficiency in plant micropropagation processes. It also provides technical consultancy for the introduction of VITROFURAL® thereby guaranteeing satisfactory results in the producer's facilities.

Produced for:

Chemical Bioactives Center. Ministry of Higher Education.

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Composition: For 100 gram:

1. Furvina 30 gr
2. Excipiente c. s.

Type of formulation: Powder.

Action: Bactericidal of wide spectrum and fungicide.

Indications: Inhibitor of the microbial contamination for means of farming used in the production of vitro plants that replaces the process of sterilization with autoclave.

Recommended concentration: 116 mg/l of Vitrofur® , meaning 35 mg/l of active principle.

Conservation: Be stored protected from the light and the moisture.

Presentation: Packing of 10 bottles of 6 g/unit.

Poisoning: Cases have not been brought.

Action Mechanism: Its action mechanism differs from current bactericidals and fungicidals. The furvina inhibits bacteria growth, inhibiting the synthesis of protein, both in vitro and in vivo. The discovery that it is a specific inhibitor of the P site of the 30S subunit promises that it will increase the arsenal capable of selectively blocking the initial steps of protein synthesis in bacteria. (Fabbretti, Brandi et al. 2012).

Fabbretti, A., L. Brandi, D. Petrelli, C. L. Pon, N. R. Castanedo, R. Medina and C. O. Gualerzi (2012).

"The antibiotic Furvina(R) targets the P-site of 30S ribosomal subunits and inhibits translation initiation displaying start- codon bias." *Nucleic Acids Res* 40(20): 10366-10374.

Advantages:

- 1- Reduces the rates of microbe contamination.
- 2- Replaces sterilization by autoclave.
- 3- Produces significant savings ($\approx 30\%$) of electrical power consumption .
- 4- Reduces amounts of agar needed by 40 -50%.
- 5- Increases productivity in the area of cultivation media and improves work conditions.
- 6- Phytotoxicity in tissues is not provoked in the concentration controlling contamination.

Instructions for use: Cultivation media should be looked after taking into account the following instructions:

1-Washing vials and lids:

This operation is done by washing vials and lids with plenty of water and then in a commercial detergent solution. The first rinse is in regular water and later with a 0.05% sodium hypochlorite solution in deionized water, and then the bottles are moved to a clean place for their immediate use.

2-Elaboration of Cultivation Media:

For the elaboration of cultivation media, the use of mother solutions and their dosage follows the same steps as those followed for media sterilized in the autoclave. After adjusting the pH of the cultivation medium, it is heated up slowly adding the gelling product until it has been completely dissolved. For solid media, the dosage of the gelling product will be between 40%-50 % of that which is used in autoclave sterilization. It is boiled for 5 minutes; it is important that the entire cultivation medium comes to a boil. To incorporate the VITROFURAL®, wait until the cultivation medium has an approximate temperature of between 80°C-85°C. We recommend to first disperse the VITROFURAL® into a small amount of the cultivation medium and then incorporate it into the rest of the medium, stirring vigorously to reach a homogenous whole and to avoid getting product sediment.

3-Essential conditions for use:

Personnel should wear sterile clothing, including caps and nose-mouth masks; they should disinfect their hands with 70 % alcohol before and after dosage, also fulfilling good production practices for working in aseptic areas. Protective goggles should be worn to avoid eye irritations in the event of splashing. After elaboration, the medium will be moved immediately to the aseptic area where the medium will be placed in doses into the cultivation vials.

4-Dosage:

The cultivation medium dosage can be done manually or mechanically. During the process stir frequently to avoid sedimentation of the VITROFURAL®, immediately putting on the lid of the vials after dosing. When using an automatic dispenser, this must be first washed with hot water (80°C-90°C) to eliminate any possible dirt that could have accumulated in the tubes. It is important to keep a maximum degree of asepsis.

5-Storage and Use:

The cultivation medium is stored in the cultivation media room inside the aseptic area. Temperature should not exceed 24°C. In the event these conditions are not available, storage should be in a cool clean place until the medium is used. It is to be used 12 hours after it has been elaborated and cooled, except in the micropropagation of potatoes when it is necessary to wait 24 hours. One should not wait for any longer than 24 hours because the product gradually degrades in the cultivation medium.

Recommendations:

1-We recommend that the product should not be inhaled or come into contact with the skin, eyes or mucous; therefore personal protective measures must be used when handling the product (smocks, goggles and nose- mouth masks).

2-Take into account that in the control and prevention of microbe contamination during the production of vitroplants, factors such as the architectural design of the work locales, provenance and the age of the initial plant, environmental hygiene or the skill and technical training of the operators are additional factors of influence. The Plant Biotechnology Institute of Villa Clara, Cuba, offers technical consultancy services which includes the experience of their specialists in the use of VITROFURAL®.

Patents: The patent for the microcidal composition product to control contamination of cultivation media for vitro-plants was granted in: Cuba No 22677, USA No 6,316,014B1, Europe 0920804, Mexico No 215995 and Brazil No PI9709932-5.

Testimonials:

The introduction of VITROFURAL® in the process of producing vitroplants in the bio-factories of the company producing and commercializing seeds for MINAG since 1999 has had great repercussions for the improvement of some basic indicators that entail optimizing the use of the workforce and humanizing this process. Among the rates showing changes, we could point to:

1-Multiplication coefficient: increased between 0.3 and 0.5 depending on the clone.

2-Contamination percentage: decreased by 2 % up to the ninth day.

3-Saving agar: 35% is saved in the cultivation medium and this means a saving of 460.00 USD per million vitroplants.

4-Consumption of electrical power: 30 % is saved because autoclaves don't have to be used for sterilization; the equivalent of approximately 330 MWh per year is saved.

5-Increased salaries due to motivation: workers have benefited since the use of VITROFURAL® involves two of the indicators that determine the amount of motivation.

6- According to our calculations, the introduction of this compound has decreased expenses to produce one million vitroplants at approximately 4000.00 USD.

Dra. Maria Adela Jiménez Velasco
Biofactories Specialist
Seed Production and Marketing Company

"Since 2004 when VITROFURAL® was introduced to the productive process of the Bioplant Center, millions of vitroplants have been produced with the use of this product, with excellent results. Some of the advantages are: increased multiplication coefficient in cultivations and decreased production costs based on the elimination of autoclaves entailing the saving of electrical power, decreased workforce in the elaboration of cultivation media, increased productivity with the use of cultivation bottles having a larger capacity, increased production capacities in cultivation chambers due to the location of cultivation vials, increasing productive efficiency of the operators of laminar flow from a norm of 1500 exp/op/pe day to 2500 exp/op/per day."

Dr. C. Oscar V. Concepción Laffitte
Associate Researcher
Bioplant Center

"Since the implementation of the use of chemical sterilization of cultivation media using VITROFURAL®, we have seen considerable saving of electrical power by replacing the use of autoclave sterilization, permitting us to save 190.5 MW. We have reduced by 46% the consumption of agar because since we no longer have to heat the cultivation medium, the stability of that substance is not altered. We confirmed that there is the possibility of a 30% increase of material in vitro since the cultivation medium is improved, the preparation of the medium is quicker and more efficient, the medium can be used 24 hours after it is prepared and the time used previously to sterilize the media in autoclaves can be used for dosing and other tasks. The contamination rates and total losses are decreased and we can achieve more effective control of the principal fungoid contaminants. This permits us to save the workforce and to humanize the preparation area for the cultivation media, since it eliminates long tiring days of being exposed to the heat coming off the autoclaves and it eliminates the risk of burning."

Ing. Claudio Pérez Bravo
CEO
Villa Clara Bio-factory

"VITROFURAL® has been used in the production of vitroplants in banana, taro, ñame and pineapple crops with excellent results. Their use has meant a noticeable decrease in the contamination rates and thus a decrease in losses of plant material. By not needing to sterilize in autoclaves, the consumption of electricity has gone down. We see a saving of 33 % in agar. With the use of VITROFURAL® we have seen no damage to plants or humans."

Ing. José Maceo Martí
CEO
Granma Bio-factory

"The use of VITROFURAL® is very important in the sterilization of cultivation media because of the advantages of this sterilization method among those we have, the growth of micro-propagated plants is stimulated, the multiplication coefficient is increased, the physical efforts of workers in the growing areas are reduced, productive capacities are augmented and total losses due to microbe contamination in the bio-factory are reduced, thereby increasing the efficiency of the productive process and causing considerable savings of gelling products and electrical power due to the fact that we don't have to use autoclaves."

MSc. Zoe Sarría Hernández
Director of Production
Plant Biotechnology Institute

Technical consultancy:

We provide technical consultancy for the modification of procedures or processes for the application of Vitrofur as a chemical disinfectant in the processes of preparing the cultivation medium for the massive propagation of plants.

Find us:

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