ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 11, pp.41271-41274, November, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

INDUCTION OF PHYTOALEXIN GLICEOLIN IN SOYBEAN BY HOMEOPATHIC MEDICINES

***Eloisa Lorenzetti and José Renato Stangarlin**

Laboratory of Phytopathology, Universidade Estadual do Oeste do Paraná – UNIOESTE, Marechal Cândido Rondon – Paraná – Brasil

ARTICLE INFO

Article History:
Received 11th August, 2016
Received in revised form
04th September, 2016
Accepted 23rd October, 2016
Published online 30th November, 2016

Key words:

Glycine max, Homoeopathy, Resistance.

ABSTRACT

The aim of this study was to evaluate the induction of phytoalexin gliceolin in soybean cotyledons by homeopathic products *Nosode* of *Macrophomina phaseolina*, *Sulphur*, *Arsenicum album* and *Sepia*. Two experiments were conducted to assess the inducing activity of phytoalexin gliceolin being the first using the homeopathic medicines *Nosode* of *Macrophomina phaseolina* and *Sulphur* and in the second test it was used the homeopathic medicines *Arsenicum album* and *Sepia*. In both assays, the boosting used were 6, 12, 24, 36 and 48CH. As additional treatment we used distilled water and hydroalcoholic solution at 30% of ethanol. The experimental design was randomized blocks, with five repetitions. The *Nosode* treatments did not induce phytoalexin synthesis, unlike Sulphur product which gave an increase of up to 85% in the production of this compound. The *Arsenicum album* treatment did not induce phytoalexin synthesis, unlike *Sepia* medicament which afforded an increase of up to 166% the production of this compound. These results indicate the potential of these homeopathic medicines in inducing phytoalexins gliceolin.

Citation: Eloisa Lorenzetti and José Renato Stangarlin, 2016. "Induction of Phytoalexin gliceolin in soybean by homeopathic medicines", *International Journal of Current Research*, 8, (11), 41271-41274.

INTRODUCTION

With a view to producing healthier agricultural products and less aggressive to the environment, man has been searching for alternative methods for disease control (Lopes *et al*., 2004; Barberato, 2002). Among the alternative mechanisms of control of plant pathogens is biological control, resistance induction and the use of products with antimicrobial activity and/or inducing resistance (Oliveira *et al*., 2011). The resistance induction in plants can be defined as a dynamic resistance, which is based on detecting physical and/or chemical barriers stimulated by the application of an inducing substance. It is a systemic or local phenomenon that has effectiveness against various pathogens, among them bacteria, fungi or viruses. Besides occurring due to localized infection, it may also be caused by treatment with microbial products or components or through organic or inorganic compounds (Bonaldo *et al*., 2005). In the literature it is possible to find numerous studies showing the efficacy of herbal extract for induction of resistance (Venturoso *et al*., 2011). Metabolites extracted from fungal mycelia have the same induction action compared to the own micro-organism that producedit (Moraes, 1992). According to Rossi (2007), homeopathic medicines can act as abiotic inducers of induced resistance, which makes them a very important alternative due to the fact that they are easy to use and have low cost.In general, one can say that plants have an arsenal consisting of several substances used for their protection, which are activated when they recognize an attack, causing changes in the metabolism of the plant cell, synthesizing this way defense proteins (Barros *et al*., 2010).It is considered the synthesis of phytoalexins (phyton = plant; alexin = compound of defense) one of the main plant defense mechanisms (Resende *et al*., 2014). It is a group of low molecular weight secondary metabolites which have antimicrobial activity, belonging to different chemical groups; among them isoflavonoids and sesquicerpenais, beyond this evidence other elicitors agents which are present can be cited as alkaloids, flavonoids, terpenoids, coumarins, sulfites, glucosides, tannins, purines, organic fatty acids (Deffune, 2001; Mazaro, 2008; Zorzete 2010). It has been reported more than 300 phytoalexins, among them gliceolin, found in soybean responsible for inhibiting activation of fungal enzymes, cytoplasmic granulation, disruption of mobile content and disruption of the plasma membrane (Gouvea *et al*., 2011).The gliceolin phytoalexin (pterocarpans) in soybeans is important in the interaction of this legume with plant pathogens, and the use of soybean cotyledons showed itself as an excellent tool for studies involving elicitor action of biotic and abiotic molecules (Schwan-Estrada *et al*., 2000).Therefore,

****Corresponding author: Eloisa Lorenzetti,***
Laboratory of Phytopathology, Universidade Estadual do Oeste do Paraná – UNIOESTE, Marechal Cândido Rondon – Paraná – Brasil

this study aimed to develop an alternative method through homeopathy to induce phytoalexins in soybean cotyledons.

MATERIALS AND METHODS

The work was divided into two trials, the first using the homeopathic remedies *Nosode* and *Sulphur* and the second using the homeopathic medicine *Sepia e Arsenicumalbum*. For the first test, *Sulphur* was acquired in a homeopathic pharmacy in the dynamizations 6CH and manipulated in 12, 24, 36 and 48CH as the Brazilian Homeopathic Pharmacopoeia (2011), diluted 1:100 and shaking 100 times and the *Nosode* of *Macrophomina phaseolina* was obtained from mycelium grown on liquid potato dextrose medium (PD), which was allowed to stir in orbital shaker at 150 rpm for 7 days, after that, it was made the fungus filtering to separate the whole culture medium, 3 g of the fungus (*Macrophomina phaseolina*) were weighed, which were placed in an amber glass along with 27 ml of cereal alcohol at 70% with the glass encased by aluminum foil and left for 21 days at rest, moving only once a day mildly to produce the mother tincture (BONATO, 2007) and through this, they were manipulated as the Brazilian Homeopathic Pharmacopoeia (2011). The dynamizations used were the same as Sulphur, 6, 12, 24, 36 and 48CH.As additional treatments we used distilled water, hydroalcoholic solution at 30% of ethanol and the suspension of *Saccharomyces cerevisiae* cells (25 mg L^{-1} of the commercial product Bio Fresh Yeast Fleishmann). Water and ethanol were used as they are solvents in the preparation of the homeopathic drugs.For the second test we used *Sepia* and *Arsenicum album* obtained in a homeopathic pharmacy in the dynamization 6CH and manipulated in 12, 24, 36 and 48CH as the Brazilian Homeopathic Pharmacopoeia (2011), diluted 1:100 and shaking 100 times. As additional treatments we also used distilled water, hydroalcoholic solution at 30% of ethanol and the suspension of *Saccharomyces cerevisiae* cells (25 mg ml^{-1} of the commercial product Bio Fresh Yeast Fleishmann).

For induction of phytoalexins in both assays we used conventional soybean seeds of the cultivar NK 412113 (conventional V-MAX).The soybean seeds (*Glycine max*) were disinfected in alcohol for 2 minutes, hypochlorite for 3 minutes and after that they were washed in distilled water until all of the hypochlorite solution (2:1) was removed. Achieved this process, the seeds were sown in trays with sand autoclaved at 120°C for 1 h. Such trays remained in the Phytopathology Laboratory of Universidade Estadual do Oeste do Paraná, for 12 days, time in which the cotyledons were recently opened. Once opened, the cotyledons were detached from the plants, washed in distilled water and wiped dry to run the test.Using a scalpel, in each cotyledon a longitudinal section has been held in their abaxial surface. In each petri dish containing filter paper moistened with sterile distilled water, we placed four cotyledons face up and over each section was applied a rate of 20 µL of the treatments in the proportion 0.1%, in other words, 100 µL for each 100 mL distilled water. For the additional treatment, *S. cerevisiae*, cell suspension was used (25 mg mL^{-1} of the commercial product Bio Fresh Yeast Fleishmann).The dishes remained incubated in BOD in the dark at 25°C for 20 h. After this period, the dishes were removed and the cotyledons from BOD transferred to film plastic bottles containing 15 ml of sterile distilled water. These flasks were shaken on an orbital shaker (150 rpm) for 1 h for the extraction of the phytoalexin formed. In spectrophotometer with absorbance of 285 nm, the reading was carried out of the supernatant. The weighing of the cotyledons after conducting the tests was performed. The tests were conducted in a completely randomized design in a factorial design with five replications. Data were submitted to analysis of variance and the means compared by Tukey test at 5% probability of error. The factor was 2 x 5 + 3, being 2 the number of drugs (in the case of the first test: *Sulphur* and *Nosode* of *M. phaseolina* and in the second test: *Sepia* and *Arsenicum album*), 5 the number of dynamizations that were used (6, 12, 24, 36 and 48CH), and 3 corresponded to the additional treatments, in other words, hydroalcoholic solution at 30% of ethanol and the suspension of *S. cerevisiae*.

RESULTS AND DISCUSSION

In the first assay for the induction of phytoalexins, the *Nosode* treatment did not induce phytoalexin synthesis, unlike *Sulphur* medicine which had its behavior represented by quadratic equation, promoting an increase of up to 85% in the production of this compound compared to the additional water treatment (Figure 1).In the second test, the treatment *Arsenicum album* did not induce the phytoalexin synthesis, contrary to *Sepia*, which had its behavior represented by quadratic equation, promoting an increase of up to 166% in the production of this compound (Figure 2).There is still no explanation of how homeopathy works, but it is believed that homeopathic medicines can act in the first instant as plants vital energy restorer through the "Law of Similars" (Souza *et al*., 2005). Therefore, one of the possible hypotheses for not having occurred significance in the treatment Sepia for phytoalexin synthesis would be the dissimilarity of the drug, the vital energy and the plant. Thus, studies are necessary to find dosages, homeopathic remedies, dynamization and frequency of appropriate application for each situation in which the plant is located. Phytoalexins form numerous defense compounds, which fit in various chemical groups. Such compounds if accumulated in sufficient levels may cause pathogen limited growth. It may be that phytoalexins act directly on the aggressor causing the death of the infected tissue (Deffune, 2001).In soybeans, gliceolin phytoalexin (pterocarpans) shown to be important in the interaction of this legume with plant pathogens (Burden; Bailey, 1975).

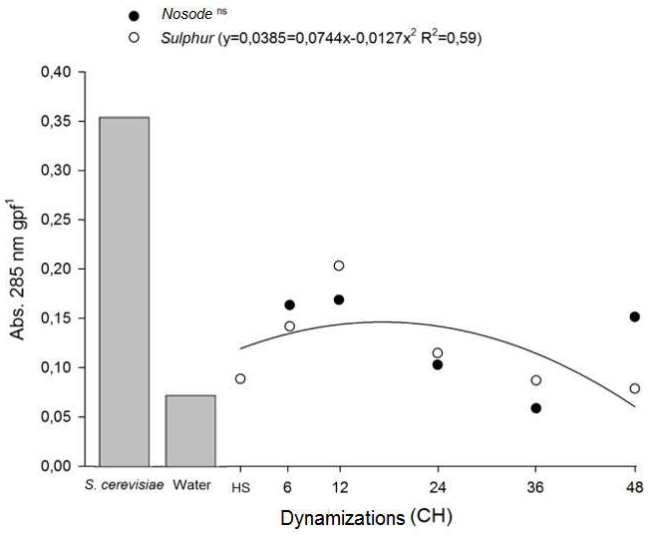

Figure 1. Induction of phytoalexins in soybean plants (*Glycine max*) treated with dynamizationsof drugs *Nosode* and *Sulphur*. ns: Absence of significance among the dynamizations of a medicinal product or between drugs. HS: hydroalcoholic solution (30% ethanol). First test

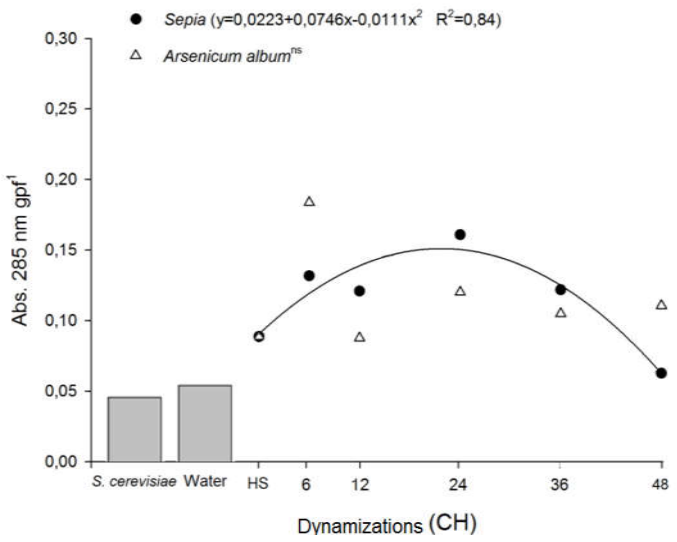

Figure 2. Induction of phytoalexins in soybean plants (*Glycine max*) treated with dynamizations of drugs *Arsenicum album* and *Sepia*. ns: Absence of significance of dynamizations of a medicinal product or between drugs. HS: hydroalcoholic solution (30% ethanol). Second test

Homeopathic medicines can contribute to physiological changes in plants, increasing not only the development but also the accumulation of secondary metabolites (Bonato *et al*., 2009). According to Duarte (2007), the use of homeopathic drugs provides a response in the metabolism of plants, causing that important bioactive compounds, depending on the given stimulation, have their levels increased or decreased. Therefore, homeopathy can be a strong option for alternative control of plant diseases (Toledo *et al*., 2011).Some studies prove the efficiency in the performance of defense mechanisms. Meinerz *et al*. (2010) showed that homeopathic drugs may be effective in inducing defense mechanisms as the peroxidase enzyme and Oliveira *et al*. (2011) of phaseolin phytoalexin in bean hypocotyls. In a study conducted by Betti *et al*. (2003), *Arsenicum album* induced resistance of tobacco plants to TMV (tobacco mosaic virus). In a study conducted by Bonato *et al*. (2009), with a view to evaluating the effect of the medicines *Sulphur* and *Arsenicum album*, in the dynamizations6, 12, 24 and 30 CH for essential oil content of mint (*Mentha arvensis* L.), found that these drugs alter the metabolism of plants, raising the number of secondary metabolites.

Conclusion

The homeopathic medicine *Sulphur* has the potential to induce phytoalexin gliceolin in soybean cotyledons, in the tested dynamizations, unlike *Nosode*.

The homeopathic medicine *Sepia* has the potential to induce phytoalexins gliceolin in soybean cotyledons, in the tested dynamizations, unlike*Arsenicumalbum*.

REFERENCES

Barberato, C. 2002. Homeopatia também na agricultura. Jornal Rural, Londrina, n. 1325, 8p.

Barros, F. C.; Sagata, É.; Ferreira, L. C. de C.; Juliatti, F. C. 2010. Indução de resistência em plantas contra fitopatógenos. Biosci. J., Uberlândia, v.26, n.2, p.231-239.

Betti, L.; Lazzarato, L.; Trebbi, G.; Brizzi, M.; Calzoni, G.L.; Borghini, G.; Nani, D. 2003. Theoretical suggestions about system variability, based on a large experimental data set. Homeopathy, v. 92, p195–202.

Bonaldo, S. M. 2005. Indução de resistência em plantas a patógenos e insetos, Piracicaba: FEALQ, p.11- 28.

Bonato, C. M. 2007. Homeopatia em Culturas Vegetais. Cultura Homeopática, n.21. p.24-28.

Bonato, C. M.; Proença, G. T.; Reis, B. 2009. Homeopathic drugs *Arsenicum album* and *Sulphur* affect the growth and essential oil content in mint (*Mentha arvensis* L.). *Acta Scientiarum. Agronomy*. v.31, p.101-105.

Burden, R. J.; Bailey, J. A. 1975. Structure of the phytoalexin from soybean. Phytochemistry, v.14, p.1389-1390.

Deffune, G. 2001. Semioquímicos, fitoalexinas e resistência sistêmica vegetal na agricultura orgânica: a explicação dos defensivos agrícolas. In: Hortibio: 1º Congresso Brasileiro de Horticultura Orgânica, Natural, Ecológica e Biodinâmica. Resumos. Botucatu-SP: Agroecológica, p.33-43.

Duarte, E. S. M. 2007. Crescimento e teor de óleo essencial em plantas de *Eucalyptus citriodora* e *Eucalyptus globulus* tratadas com homeopatia. Tese (Doutorado-Área de Concentração em Fitotecnia) - Departamento de Fitotecnia, Universidade Federal de Viçosa, Viçosa, 188p.

Farmacopéia homeopática brasileira. 2011. 2. ed.: Editora Atheneu, São Paulo – SP.

Gouvea, A.; Zanotti, J.; Luckmann, D.; Pizzatto, M.; Mazaro, S. M.; Possenti, J. C. 2011. Efeito de extratos vegetais em soja sob condições de laboratório e campo. Rev. Bras. De Agroecologia, v.6, n.2, p.70-78.

Lopes, P. S. N.; Leite, G. L. D.; Sá, V. G. M.; Silva, A. C.; Soares, M. A. 2004. Controle fitossanitrio alternativo em comunidades de pequenos produtores rurais no Norte de Minas Gerais. Anais do 2º Congresso Brasileiro de Extensão Universitária, Belo Horizonte; p.16-22.

Mazaro, S. M.; Citadin, I.; Gouvêa, A. de; Luckmann, D.; Guimarães, S. S. 2008. Indução de fitoalexinas em cotilédones de soja em resposta a derivados de folhas de pitangueira. Ciência Rural, v.38, n.7, p.1824 -1829.

Meinerz, C. C.; Gheller, D.; Toledo, M. V.; Muller, S. F.; Stangarlin, J. R. 2010. Atividade de peroxidase na indução de resistência de tomateirocontra *Alternaria solani* por medicamentos homeopáticos. Anais do XIX Encontro Anual de Iniciação Científica. p.1-4.

Moraes, W. B. C. 1992. Controle alternativo de fitopatógenos. Pes. Agropec. Bras,v.27, S/N, p.175-190.

Oliveira, J. S. B.; Maia, A. J.; Schwan-estrada, K. R. F.; Carneiro, S. M. De T. P. G.; Bonato, C. M. 2011. Indução de fitoalexinas em hipocótilos de feijoeiro por preparados homeopáticos de *Eucalyptus citriodora*. Cadernos de Agroecologia, v.6, n.2. p.1-5.

Resende, M. L. V. 2014. Mecanismos de defesa de plantas a patógenos. In: Zambolim, L.; Jesus Júnior, W. C.; Rodrigues, F. A. (Org.). 2014. O essencial da fitopatologia: epidemiologia de doenças de plantas. São Carlos: Suprema, v.1, p.187-210.

Rossi, F.; Arévalo, R. A.; Ambrosano, E. J.; Guirado, N.; Ambrosano, G. M. B.; Mendes, P. C. D.; Mota, B.; Atzingen, E. M. M. V.; Menuzzo, M. M.; Varella, A. S. 2007. Aplicação de preparado homeopártico no controle da tiririca em área agroecológica. Rev. Bras. de Agro. v.2, n.1, p.870-873.

Schwan-estrada, K. R. F.; Stangarlin, J. R.; Cruz, M.E. S. 2000. Uso de extratos vegetais no controle de fungos fitopatogênicos. Floresta, v.30, n.1-2, p.129-137.

Souza, A. F.; Collet, M. A; Bonano, C. M. 2005. Efeito de soluções homeopáticas no controle da ferrugem (*Phakopsora euvitis* Ono) em videira. Arq. Apadec v.9, n.2, p.27-30.

Toledo, M. V.; Stangarlin, J. R.; Bonato, C. M. 2011. Homeopathy for the control of plant pathogens. In: Méndez-vilas, A. 2011. Science against microbial pathogens: communicating current research and technological advances. Badajoz: Formatex. p.1063-1067.

Venturoso, L. R.; Bacchi, L. M. A.; Gavassoni, W. L. 2011. Atividade antifúngica de extratos vegetais sobre o desenvolvimento de fitopatógenos. Summa Phytopathologica, v.37, n.1, p.18-23.

Zorzete, P. 2010. Fungos, Micotoxinas e Fitoalexina em Variedades de Amendoim do Plantio ao Armazenamento. Tese (Doutorado em Microbiologia) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 188p.

****