

# An In Vitro Evaluation Of Antagonistic Activity Of *Secale Cornutum* Against Phytopathogenic Fungus *Claviceps Purpurea*

Bapi Mondal<sup>1</sup>, Anupam Mukherjee<sup>2</sup>, Bipinraj Nirichan Kunchiraman<sup>3</sup>, Suresh Jagtap<sup>4</sup>, Varsha Umesh Ghathe<sup>5\*</sup>

<sup>1</sup>Department of Homoeopathic Pharmacy, Homoeopathic Medical College and Hospital, Bharati Vidyapeeth (DTU), Pune, Maharashtra, India.

<sup>2</sup>West Bengal Homoeopathic Health Service, Directorate of Homoeopathy, Department of Health and Family Welfare, Government of West Bengal, West Bengal, India.

<sup>3</sup>Department of Microbiology, Rajiv Gandhi Institute of Information Technology and Biotechnology, Bharati Vidyapeeth (DTU), Pune, Maharashtra, India.

<sup>4</sup>Department of Herbal Biotechnology, Interactive Research School for Health Affairs, Bharati Vidyapeeth (DTU) Medical College Campus, Pune, Maharashtra, India.

<sup>5\*</sup>Department of Homoeopathic Pharmacy, Homoeopathic Medical College and Hospital, Bharati Vidyapeeth (DTU), Pune, Maharashtra, India.

\*Corresponding Author: - Varsha Umesh Ghathe

\*Ph.D., Department of Homoeopathic Pharmacy, Homoeopathic Medical College and Hospital, Bharati Vidyapeeth (DTU), Pune, Maharashtra, India. Email ID- varshaghathe29@gmail.com, varsha.ghate@bharativedyapeeth.edu

DOI: 10.47750/pnr.2023.14.03.13

## Abstract

The India is an agriculture-based country, infection by microorganisms such as fungi, bacteria, or other lower-class organisms is a major issue for obtaining a high-quality crop, and it will have an impact on both the quality and quantity of production. The ergot is one of the common diseases seen in cereal crop caused by fungus *Claviceps purpurea*, and *Secale cornutum* is a homoeopathic medicine prepared from *Claviceps purpurea* fungus infected rye plants. Homoeopathy is a science originated on the principle of "Similia Similibus Curenture" and by application of this principle; authors have conducted *in vitro* study with an aim to check the efficacy of the homoeopathic drug *Secale cornutum* against the fungal infection *Claviceps purpurea*. Potato Dextrose Agar (PDA) mixed with distilled water and slightly heated the mixture through water bath to mix it well, then 13 PDA agar plate was prepared, out of which 4 duplicate plates were prepared for various potencies (Q, 30C, 200C, 1M) of Hom. Medicine *Secale cornutum*. Four agar plates and their four duplicates were treated with homoeopathic medicine *Secale cornutum* with potencies of Q, 30C, 200C, 1M. A serial dilution of active control fungicide crapton was prepared with 1ml of water of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  out of which three agar plates were treated with serial dilution of active control of  $10^{-1}$ ,  $10^{-3}$ , and  $10^{-6}$ . One plate was treated with alcohol and one plate for negative control. Optimum density (OD) of fungus culture was prepared and 50 mules of 1 OD was diffused with each agar plate. To observe the zone of inhibition three wells of 8mm diameter were inserted in each plate, and kept those at 25°C in incubator for 72 hrs. In this research, only the serial dilutions of active control crapton  $10^{-1}$  and  $10^{-3}$  showed zones of inhibition of 38mm and 26mm, respectively, but none of the potencies (Q, 30C, 200C, 1M) of homoeopathic medicine *Secale cornutum*, showed any zone of inhibition. Inhibition and /or control of the infection using of homoeopathic medicine was our prime objective so we have explored antifungal activity of various potency Q, 30C, 200C, and 1M of *Secale cornutum*. It will take more researches to find out another homoeopathic medicine having antifungal activity on pathogenesis of *Claviceps purpurea*.

**Keywords:** *Claviceps purpurea*, *Secale cornutum*; in- vitro; Antifungal activity; Homoeopathy.

## 1. INTRODUCTION

Agro-homoeopathy is a new approach in agricultural research for higher crop productivity and protection of plants against various stress conditions. [1] Homoeopathic medicines can be used for various purposes in agriculture from seed germination to crop production. Initial work began by observing the effect of homoeopathic medicines on seed germination of various crop species. From late 20th century, logical thinks about are performed swiftly in research facilities. The think about of agro-homoeopathy isn't restricted as it were up to seed germination. Destruction of biotic and abiotic stresses was moreover made conceivable by the hone of Homoeopathy in agriculture.

In 1986, V. D. Kaviraj to begin with utilized *Belladonna* effectively to treat an infected apple tree. Here he opened a modern entryway to rural inquire about. Agro-homoeopathy don't replace the compost within the agroecosystem. The homoeopathic treatment to plant gives solid but transitory incitement to re-establish framework mineral insufficiencies

and to progress assimilation and mineral digestion system in plants. It can alter phytochemical profile and soil wellbeing to bring back ordinary wellbeing of the plants. Nano-scale concentration of homeopathic medicate works for plant health too. [2] The India is an agriculture-based nation, farming could be a critical source of income in India, and it bears a noteworthy financial burden in arrange to supply a business. [3] Infection by microorganisms such as fungi, bacteria, or other lower-class organisms is a major issue for obtaining a high-quality crop, and it will have an impact on both the quality and quantity of production. [4] The essential crops developed and devoured in India are wheat, rye, rice, jowar, and maze, which are infected by numerous microorganisms but *Claviceps purpurea* is one of them. [5, 6] It infects plant seeds and eating of contaminated seeds by human being causes the disease ergotism. [7] However, there is no specific fungicide or chemical agent available to combat *Claviceps purpurea* infection. [8] Utilize of homeopathic medication to control and avoid different diseases of plant caused by microorganism isn't as it were be cost-effective but it moreover decreases the chances of chemical toxification deliver from abuse of agrochemicals (i.e., fertilizer, fungicide, bug spray) and advance the concept of natural cultivating as most of homeopathic medication are prepared from natural sources. *Secale cornutum* (SC) is a homeopathic medicine prepared from *Claviceps purpurea* fungus infected rye plants. [9] Homoeopathy is a science originated on the principle of *Similia Similibus Curenture*. [10] and by application of this principal authors have conducted *in vitro* study with an aim to check the efficacy of the homeopathic drug SC against the fungal infection *Claviceps purpurea*. *In vitro* study is the experiment to be done under certain controlled environment, such as a test tube or petri plates. Saving of time and money can be accomplished with such pre-clinical studies. Further it aids the researchers in coming to a primary conclusion based on their findings. It also provides a heads-up regarding the constraints and risks of further research.

## 2. MATERIAL

### 2.1. Collection of the fungus culture

*Claviceps purpurea* [NCIM no. 1046 CMI 44613 (1972)] was collected from National Collection of Industrial Microorganism (NCIM), of CSIR-National Chemical Laboratory (NCL) Pune-411008, Maharashtra, India.

### 2.2. Procuring of the Homoeopathic medicine

The Homoeopathic medicinal product *Secale cornutum* in various potencies (Q, 30CH, 200CH, 1M) were procured from a GMP certified homoeopathic manufacturing unit, vide; Dr. Willmar Schwabe India Pvt. Ltd., Noida-201304, Uttar Pradesh, India.

### 2.3. Active control

Active Chemical Control Crapton Fungicide (N-Trichloromethylthio~cyclohexene-1, 2- dicarboximide fungicide) was procured from Naik Krishi Udyog & Co, Swargate, Pune- 411002, Maharashtra, India.

## 3. METHODS

### 3.1 Preparation of Mother Culture

1.95gm of Potato Dextrose Broth (PDB) was mixed with 150 ml of distilled water and slightly heated the mixture through water bath to mix it well.

### 3.2 Calculation for PDB

PDB need for 1000ml water x Quantity of water ÷ 1000ml water [13 mg x 150ml distilled water ÷ 1000 ml water] = 1.95 mg of PDB. After that the solution was divided in 30ml equally and poured in five (05) inoculation tube. A loop full of *Claviceps purpurea* culture was taken and inoculated in the broth. And kept it at 25°C in incubation for 72 hrs.

### 3.3 Well diffusion method

The antifungal activity of homoeopathic medicine *Secale cornutum* with its various potencies (Q, 30CH, 200CH, 1M) was assessed through well diffusion method.

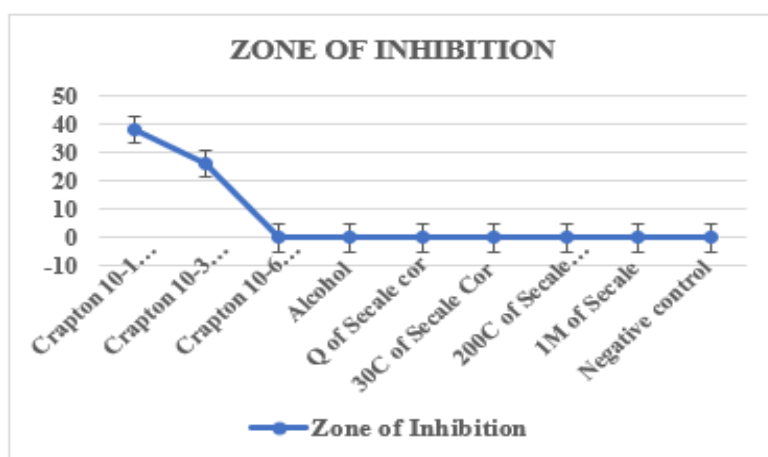
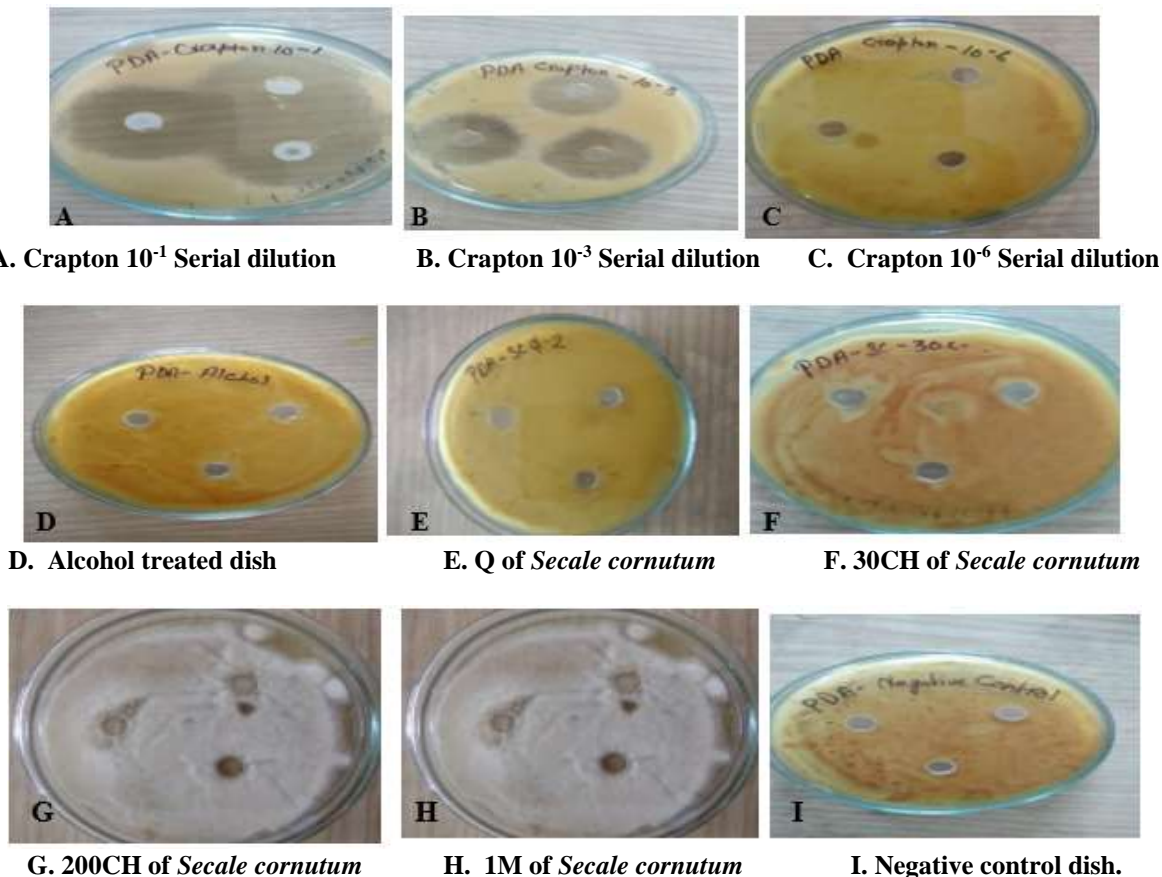
### 3.4 Calculation for PDA

(PDA need for 1000 ml of water) x (Quantity of water) ÷ 1000 ml water [39 mg x 300ml distilled water ÷ 1000 ml water] = 11.7 mg of PDA. Calculated 11.7 gm of Potato Dextrose Agar (PDA) was mixed with 300 ml of distilled water then slightly heated the mixture through water bath to mix it well, then 13 PDA agar plate was prepared, out of which 4 duplicate plates were prepared for various potencies (Q, 30CH, 200CH, 1M) of homoeopathic medicine SC. Four agar plates and their four duplicates were treated with homoeopathic medicine SC with potencies of Q, 30CH, 200CH, 1M. A serial dilution of active control fungicide- Crapton was prepared with 1ml of water of 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> and three agar plates were treated with serial dilution of active control of 10<sup>-1</sup>, 10<sup>-3</sup>, and 10<sup>-6</sup>. One plate was treated with alcohol and one plate for negative control. Optimum density (OD) of fungus culture was prepared and 50 mules of 1 OD was diffused with each agar plate. To observe the zone of inhibition three wells of 8 mm diameter were inserted in each plate, and kept those at 25°C in incubation for 72 hrs.

## 4. RESULT

In this study, serial dilutions of active control Crapton  $10^{-1}$  and  $10^{-3}$  showed Zones of inhibition of 38 mm and 26 mm, respectively, but no Zone of inhibition was seen in  $10^{-6}$  Crapton, Ethayl alcohol and various potencies of homoeopathic medicine *Secale cornutum* i.e., Q, 30CH, 200CH, 1M.

**Fig. 1:** *Claviceps purpurea* inoculated plate treated with various dilution of Crapton and various potencies of *Secale cornutum*.



**Fig. 2:** Graphical presentation of Zone of inhibition.

#### 4.2 Statistical analysis

All the data was recorded and tested statistically with the one way analysis of variance (ANOVA) to compare between the control and target groups. The impression of the result F test statistics in the ANOVA table is greater (4.069) than the F critical value (3.1789). So, author accepted the null hypothesis that *Secale cornutum* Q, 30CH, 200CH, and 1M not shown antifungal activity on *Claviceps Purpure*.

## 5. DISCUSSION

The primary crops cultivated and consumed in India are wheat, rye, rice, jowar, and maize, which are infected by many microorganisms and *Claviceps purpurea* is one of them.<sup>[6-8]</sup> It infects plant seeds, and eating of infected seeds by humans or other higher animals causes the disease ergotism. However, there is no specific fungicide or chemical agent available to combat *Claviceps purpurea* infection.<sup>[8]</sup> Use of homeopathic medicine to control and prevent various diseases of plants caused by microorganisms will be not only cost-effective but it may reduce the chances of chemical toxication produced from overuse of agrochemicals (i.e., fertilizer, fungicide, insecticide). *Secale cornutum* is a homeopathic medicine prepared from *Claviceps purpurea* fungus infected rye plants,<sup>[9, 10]</sup> but there is no specific research on the efficacy of *Secale cornutum* on pathogen infection. *In vitro* study the experiment done under certain controlled environment, such as a test tube or petri dish.<sup>[11, 12]</sup> It not only helps the researchers to draw a primary conclusion based on its finding, also it is cost effective and time saving.<sup>[13, 14]</sup> It also gives a prior idea about limitation, hazards of further studies.<sup>[14, 15]</sup> So, research had been carried out with aim to establish an *in vitro* evaluation of antagonistic activity of SC against phytopathogenic fungus *Claviceps purpurea*. In this study only serial dilutions of active control Crapton 10<sup>-1</sup> and 10<sup>-3</sup> showed zones of inhibition of 38mm and 26mm, respectively, but none of the potencies of SC i.e.; Q, 30CH, 200CH and 1M, showed any zone of inhibition. As a result, we accept the null hypothesis that Homeopathic medicine *Secale cornutum* with potencies of Q, 30CH, 200CH, and 1M does not have antifungal activity on *Claviceps purpurea*.

## 6. CONCLUSION

*Claviceps purpurea* infection of crop is a major problem and consumption of those infected crop by higher animal caused ergotism. Inhibition and/or control of the infection using of homeopathic medicine was our prime objective so we have explored antifungal activity of various potency Q, 30CH, 200CH, and 1M of *Secale cornutum*. It will take more researches to find out another homeopathic medicine having antifungal activity on pathogenesis of *Claviceps purpurea*.

### Conflict Of Interest

The author declares no conflict of interest.

## ACKNOWLEDGMENT

The work was completed as part of the STSH project under the auspices of the Central Council for Research in Homeopathy, New Delhi, under Ministry of AYUSH, Government of India for which the authors are grateful. We would like to convey our sincere appreciation and debt of gratitude to Prof. (Dr.) Avinash R. Mhetre, I/C Principal at the BV (DTU)-HMC, Pune. Further we are thankful to Prof. (Dr.) Anita S. Patil, Dean (Faculty of Homeopathy) and PG-Coordinator at the BV (DTU)-HMC, Pune for individual invaluable encouragement and support in study.

## REFERANCE

- Lorenzo, F. D., Dinelli, G., Marotti, I., & Trebbi, G. (2021). Systemic Agro-Homeopathy: A New Approach to Agriculture. *OBM Integrative and Complementary Medicine*, 06(03), 1–1. <https://doi.org/10.21926/obm.icm.2103020>
- Kaviraj VD, (2011) Homeopathy for Farms and Gardens, Narayana Publishers, Second revised edition.
- Gupta, D., Vyas, D., & Sharma, D. (2020, November). Agrohomoepathy-Does homeopathy have role in agriculture. *Wesleyan journal of research*, 13(40).
- Welfare, M. o. (2019). Agricultural Statistics at a glance 2018. Department of Agriculture, Cooperation & Farmers Welfare Directorate of Economics and Statistics. Delhi, India: Government of India. Retrieved from <http://eands.dacnet.nic.in>
- Saini R.K. (2014). Novel approaches in pest and pesticide management in Agro ecosystem.
- Reddy, E. S., & Bembrekar, S. K. (2017, May). A survey of some common crop diseases from kinwat. *international journal for innovative research in multidisciplinary field*, 3(5), 95-96.
- Jain, S., Tripathi, S., Nety, S., Sen, C., Debbarma, B., & Koley, K. (2021). Alkaloid poisoning in cattle exposed to kodo millet. *The Pharma Innovation Journal*, 351-352. Retrieved from <https://www.thepharmajournal.com/>
- Schumann, G. L., & Uppala, S. S. (2000). The American Phytopathological Society (APS). doi:10.1094/PHI-I-2000-1016-01
- Saini R.K. (2014). Novel approaches in pest and pesticide management in Agro ecosystem. Ministry of Health and Family Welfare, G. o. (1971). *Homeopathic Pharmacopoeia Of India (H.P.I.) (Vol. 1)*. Delhi, India: Ministry of Health and Family Welfare, Govt. of India.
- Hahnemann, S. (1921). *Organon of Medicine* (6th ed.). (W. Boericke, Trans.) B.Jain Publishers (P) Ltd.
- J. G. Menzies & T. K. Turkington (2015) An overview of the ergot (*Claviceps purpurea*) issue in western Canada: challenges and solutions, *Canadian Journal of Plant Pathology*, 37:1, 40-51, DOI: 10.1080/07060661.2014.986527
- Blaney Barry J., Molloy John B., Brock Ian J. (2009) Alkaloids in Australian rye ergot (*Claviceps purpurea*) sclerotia: implications for food and stockfeed regulations. *Animal Production Science* 49, 975-982. <https://doi.org/10.1071/AN09030>
- W. A. Taber and L. C. Vining. The influence of certain factors on the in vitro production of ergot alkaloids by *Claviceps purpurea* (fr.) Tul. *Canadian Journal of Microbiology*. 4(6): 611-626. <https://doi.org/10.1139/m58-067>
- Hicks, C., Witte, T. E., Sproule, A., Lee, T., Shoukouhi, P., Popovic, Z., Menzies, J. G., Boddy, C. N., Liu, M., & Overy, D. P. (2021). Evolution of the Ergot Alkaloid Biosynthetic Gene Cluster Results in Divergent Mycotoxin Profiles in *Claviceps purpurea* Sclerotia. *Toxins*, 13(12), 861. <https://doi.org/10.3390/toxins13120861>
- Hirsch, C., & Schildknecht, S. (2019). In Vitro Research Reproducibility: Keeping Up High Standards. *Frontiers in Pharmacology*, 10. <https://doi.org/10.3389/fphar.2019.01484>