International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 10, Issue11-2023

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.11.006

Effect of the Fungicide Bavistin on *in vitro* Germination of Pollen of *Catharanthus roseus* (L.) G. Don. and *Clitoria ternatea* L.

[1]Saloni Bahri, [1]Madhu Bajaj*, [2]Renuka Agrawal, [2]Somdutta Sinha Roy, [3]Mishthi Khurana and [3]Yamini Sharma

[1]Associate Professor, Department of Botany, Miranda House, University of Delhi, Delhi 110007
[2] Assistant Professor, Department of Botany, Miranda House, University of Delhi, Delhi 110007
[3]B.Sc. Life Sciences, Hansraj College, University of Delhi, Delhi 110007
*Corresponding author: [1]*madhu.bajaj@mirandahouse.ac.in*

Abstract

Crop plants under cultivation are prone to be destroyed by fungal diseases. Application of fungicides on crop plants to save them from fungal pathogens is a common practice. Many of the chemicals have been reported to be toxic through the *in vitro* pollen germination assay. Pollen grains offer a sensitive and easy system for assessment of cytotoxicity of chemicals. Pollen grains can be cultured in germination medium in which the test chemical has been incorporated. Inhibition of pollen germination and pollen tube elongation are used as the parameters that indicate cytotoxicity. In the current study, the cytotoxicity of Bavistin, a common fungicide, was evaluated by studying its effect on *in vitro* pollen germination and pollen tube growth in two plant species namely, *Catharanthus roseus* (L.) G. Don. and *Clitoria ternatea* L. Germination media with four dilutions (1:1, 1:2, 1:4 and 1:8) of the recommended dose of Bavistin were used to culture pollen grains. The results demonstrate that all tested dilutions of Bavistin are cytotoxic. Thus, the fungicide should be used with caution.

Keywords: Bavistin, cytotoxicity, pollen grains, *in vitro* pollen germination, pollen tube length

Introduction

Crop plants grown under field conditions are susceptible to many fungal pathogens which cause diseases resulting in heavy losses of productivity. For sustainable cultivation and protection of crops from fungal pathogens several agrochemicals such as fungicides are utilized.

Fungicides mostly act by damaging fungal cell membranes or interfering with fungal cell metabolism and thus prevent the growth of fungi and their spores (npic portal). They are commonly applied as seed treatment and foliar spray. Seed-applied fungicides for treating soil-borne fungi

may persist at low concentrations for long periods in the plant or the rhizosphere; many fungicides are systemic and penetrate into and are translocated in plant tissues (Zubrod et al., 2019). Although an effective remedy, because of their frequent application fungicides are a cause of pollution of environment due to toxic residue and also lead to development of fungal resistance (Yoon et al., 2013). Fungicides can enter aquatic ecosystems where they are toxic to non-target organisms (Zubrod et al., 2019). The timing of their application often coincides with pollen germination. Spray application done on the flowers to obtain better yield may hinder the pollen tube formation and thus the process of fertilization (Kargar and Imani, 2011).

Seeds as well as pollen grains can serve as sensitive and reliable assay systems for toxicological studies (Subramanyan et al., 2023). Pollen grains have been used in studying the effects of fungicides, herbicides, insecticides, pesticides, pollutants and toxic substances, and in biomonitoring air pollution caused by heavy metals (Gentile et al., 1971; 1973; 1978; Bilderback, 1981; Sutherland et al., 1984; Nikolov et al., 2000; Kamble, 2005; Mehri et al., 2006; Salgare, 2006; Meshram and Chaturvedi, 2017). Fungicides considered to be non-toxic for plants in field trials have been found to be cytotoxic for male gametophyte development (Pavlík and Jandurová, 2000). It is essential to screen different pesticides for their effect on pollen grain germination before their application in the fields since chemical pesticides can be very harmful (Padilla et al., 2017). *In vitro* pollen germination and pollen tube growth are easy and sensitive methods for assessment of the effects of toxic compounds and monitoring pollution (Shivanna and Rangaswamy, 1992; Shivanna, 2003). These methods do not require aseptic conditions and their results are rapid. Inhibition of pollen germination and pollen tube elongation in presence of a toxic compound in *in vitro* pollen cultures may be considered indicative of its cytotoxicity (Pavlík and Jandurová, 2000; Subramanyan et al., 2023).

In the present investigation, toxic effect of Bavistin was studied on pollen grains of two plant species, *Catharanthus roseus* (L.) G. Don. and *Clitoria ternatea* L. using reduction of pollen germination and pollen tube elongation as parameters of toxicity. *C. roseus*, common name 'Madagascar Periwinkle', is a medicinal plant that belongs to family Apocynaceae while *C. ternatea*, a member of family Fabaceae and commonly known as 'Butterfly pea', is used in traditional Ayurvedic medicine. The pollen grains of *Catharanthus roseus* are spheroidal and tricolporate (PalDat - Palynological Database A). *Clitoria ternatea* has triangular and tricolpate pollen grains (PalDat - Palynological Database B). The pollen grains of both flowers are monads and large in size (51-100µm).

Bavistin (Carbendazim 50 % WP) is a broadspectrum systemic fungicide used to control various fungal plant diseases such as blight, mildew, mold, spot and rot (Ghurde et al., 2021). Bavistin has also been utilised in tissue culture studies for surface sterilization of explants and for shoot regeneration (Bantawa et al., 2009; Preethi et al., 2011; Reddy et al., 2012; Tiwari et al., 2012; Kumar et al., 2019; Chopra et al., 2022).

Materials and Methods

Standardisation of pollen germination medium

The flower buds of *C. roseus* and *C. ternatea* were picked from the garden in the morning and kept under a table lamp to facilitate anthesis and anther dehiscence. The pollen grains were collected from freshly dehisced anthers and pollen germination medium was standardised by checking their germinability in solutions of 20% sucrose, 30% sucrose or Brewbaker and Kwack medium (1964) at ambient room temperature (30-33^{o}C) by raising hanging drop cultures of pollen grains. The best percent germination was obtained in modified Brewbaker and Kwack medium (with 30% sucrose rather than the suggested 10%) after 60 minutes of incubation in the medium and further studies were carried out in this medium.

Effects of different concentrations of Bavistin on pollen germination and pollen tube elongation

A 0.001% aqueous stock solution of commercially available Bavistin was prepared. Appropriate amount of the stock solution was incorporated into the modified Brewbaker and Kwack germination medium to get pollen germination medium containing 1:1, 1:2, 1:4 and 1:8 dilutions of Bavistin volume/volume. Hanging drop cultures of pollen grains were raised in germination medium containing Bavistin solution as well as control (germination medium without fungicide).

Raising hanging drop cultures of pollen grains

To set up hanging drop cultures, a drop of the germination medium was placed on the lid of a cavity block and the edges of the lid were lined with vaseline. A few pollen grains were dusted on the drop of medium and spread uniformly with the help of a needle. The lid with the culture drop was inverted over the cavity of the cavity block. After incubation for 60 minutes pollen grains were scored for percent germination in three random non-overlapping microscopic fields. A pollen grain was considered as germinated only when the pollen tube length was equal to or exceeded the diameter of the pollen grain. An ocular micrometer was used to measure the length of the pollen tubes. Pollen grains whose tubes had burst at their tips were not scored. All cultures were repeated in triplicate and the average values were calculated for interpreting the results.

Results

Pollen grains of both the plant species germinated well in modified Brewbaker and Kwack medium. *C. roseus* showed higher germinability (91.22%) in the germination medium than the pollen of *C. ternatea* (44.57%). All the dilutions of Bavistin tested resulted in inhibition of pollen germination in both the species in a concentration dependent manner (Figures1 and 2). Most of the ungerminated pollen grains were found to be hydrated and many produced papillae. The average inhibition of pollen germination ranged from 21.00% (1:8 dilution) to 65.11% (1:1 dilution) for *C. roseus* while the values ranged from 42.88% (1:8 dilution) to 96.9% (1:1 dilution) for *C. ternatea* (Table 1; Figure 3).

Table 1. Effect of Bavistin on germination of *C. roseus* and *C. ternatea* pollen.

Dilution of Bavistin	*Catharanthus roseus*		*Clitoria ternatea*	
	Average % pollen germination	%Pollen germination inhibition	Average % pollen germination	% Pollen germination inhibition
0 (Control)	91.22	0	44.57	0
1:8	72.06	21.00	25.46	42.88
1:4	46.55	48.97	22.65	49.19
1:2	46.00	49.57	13.62	69.44
1:1	31.83	65.11	1.38	96.90

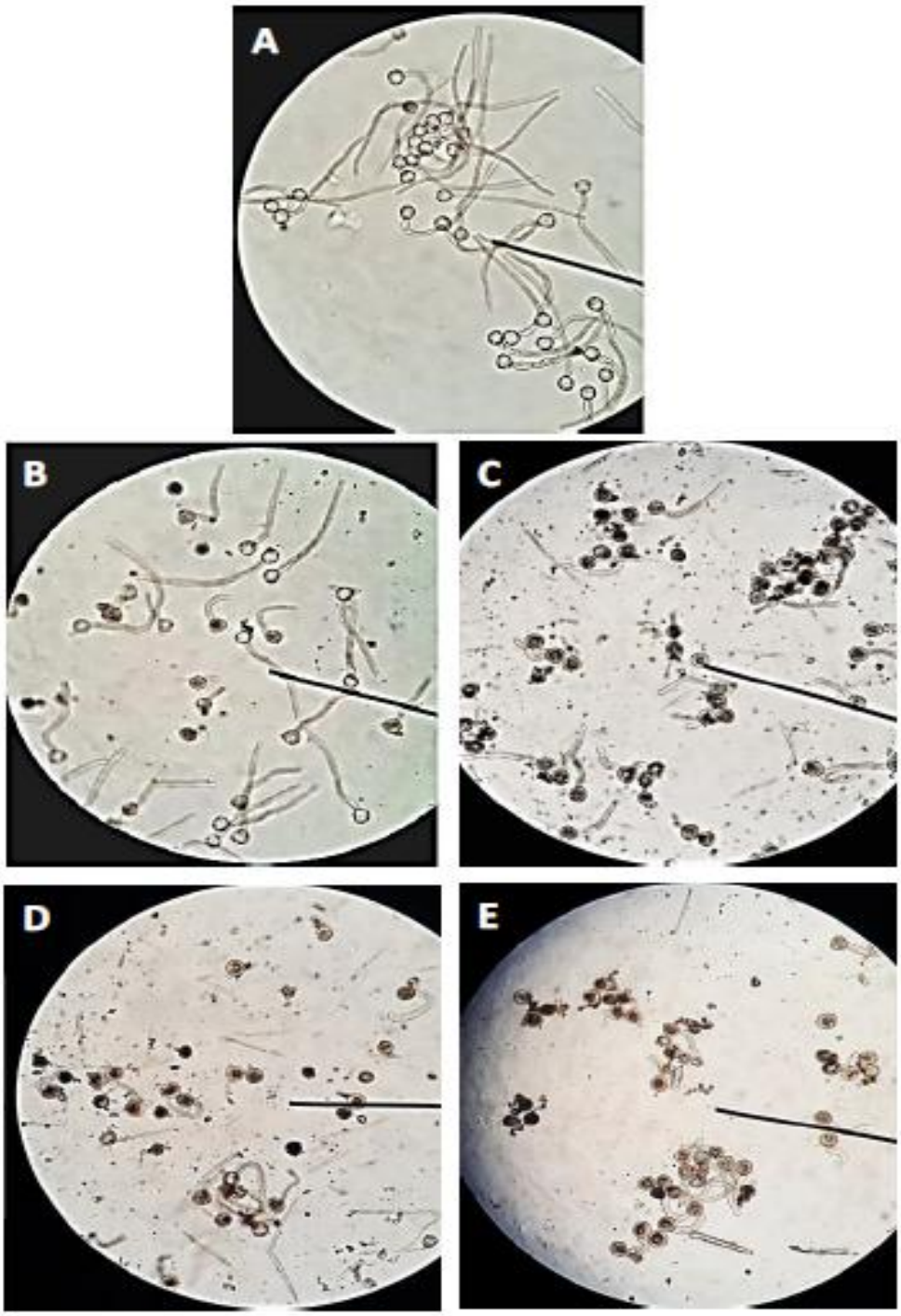

Figure 1. Micrographs of 60 minutes old cultures showing effect of Bavistin on pollen germination and pollen tube length of *Catharanthus roseus.* A. Control in modified Brewbaker and Kwack medium; B to E: In 1:8, 1:4, 1:2 and 1:1 dilution of Bavistin, respectively.

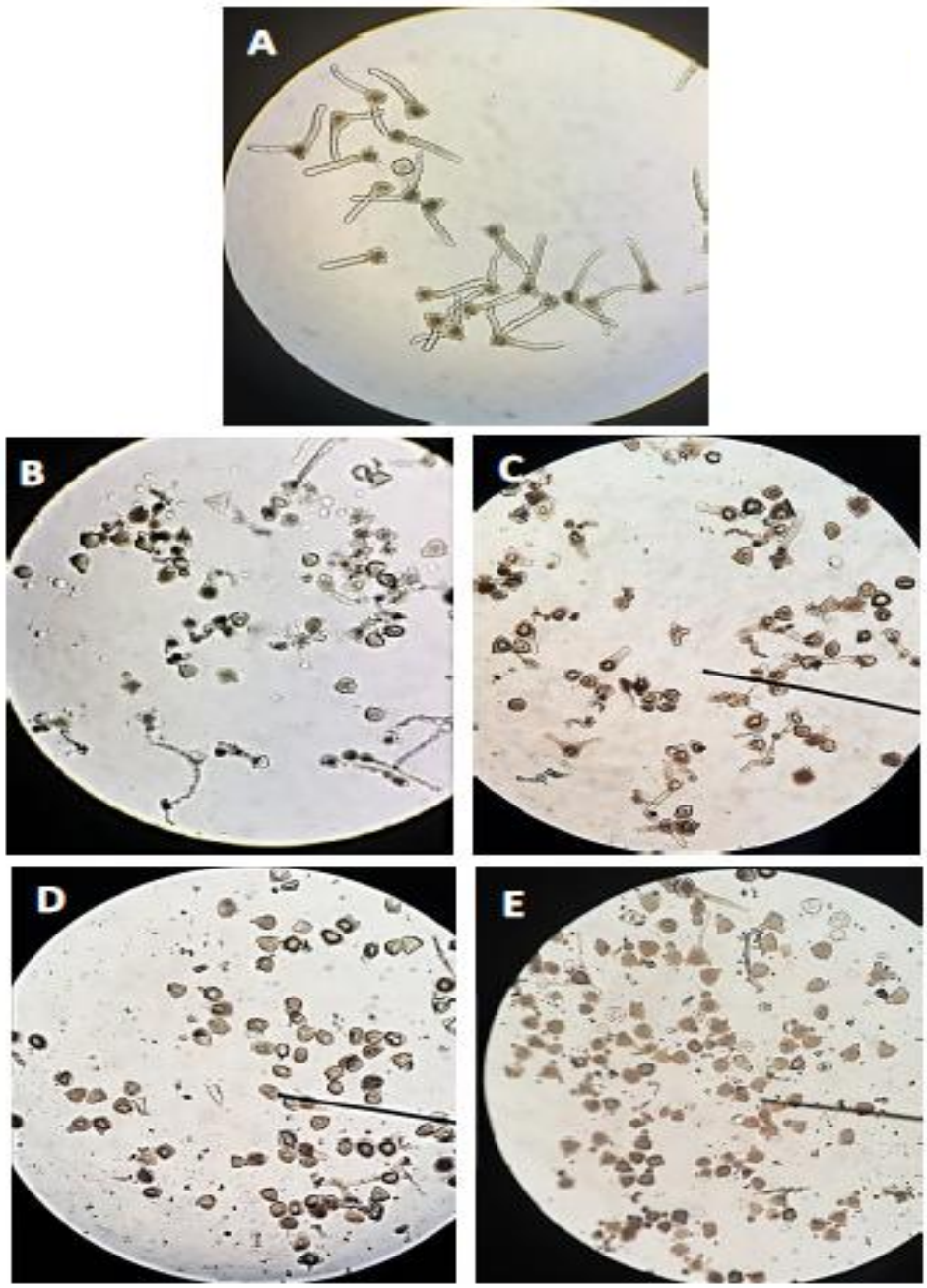

Figure 2. Micrographs of 60 minutes old cultures showing effect of Bavistin on pollen germination and pollen tube length of *Clitoria ternatea.* A. Control in modified Brewbaker and Kwack medium; B to E: In 1:8, 1:4, 1:2 and 1:1 dilution of Bavistin, respectively.

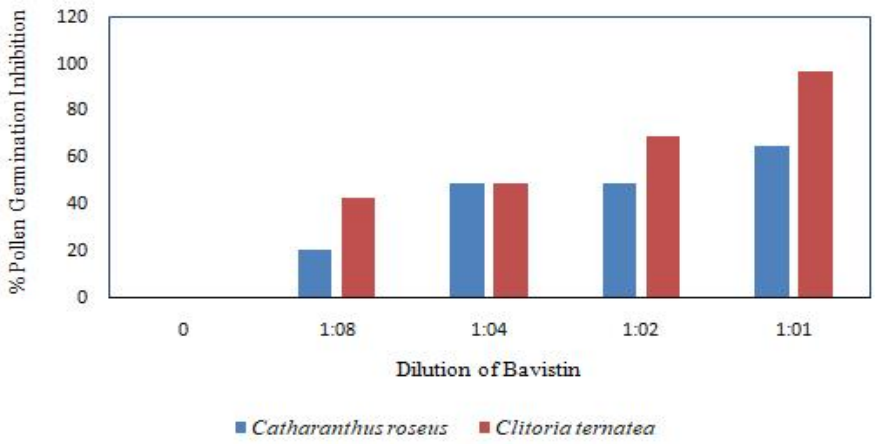

Figure 3. Inhibition of pollen germination by Bavistin

More than one pollen tube was produced by some pollen grains in both species as the pollen grains of *C. roseus* are tricolporate and those of *C. ternatea* are tricolpate. Like percent pollen germination, the pollen tube length was longer in the modified Brewbaker and Kwack medium in case of *C. roseus* (467µm) than *C. ternatea* (198.16µm). While the average length of *C. roseus* pollen tube decreased with increase in concentration of Bavistin, the same trend was not apparent for *C. ternatea* (Table 2). The average % pollen tube length inhibition in the former ranged from 26.12% (1:8 dilution) to 47.75% (1:1 dilution) whereas in the latter a wider range of 32.21% (1:8 dilution) to 81.33% (1:1 dilution) was observed (Table 2; Figure 4). Thus, the inhibition of pollen tube length was extremely high with 1:1 dilution of Bavistin in case of *C. ternatea* compared to *C. roseus*. In both species at higher concentrations of Bavistin (1:2 and 1:1 dilution) pollen grains produced mostly papillae which did not elongate into pollen tubes (Figures 1 and 2).

Table 2. Effect of Bavistin on germination of *C. roseus* and *C. ternatea* pollen tube length.

Dilution of Bavistin	*Catharanthus roseus*		*Clitoria ternatea*	
	Average pollen tube length (µm)	% Pollen tube length inhibition	Average pollen tube length (µm)	% Pollen tube length inhibition
0 (Control)	467.00	0	198.16	0
1:8	345.00	26.12	134.33	32.21
1:4	322.00	31.05	155.50	21.53
1:2	279.00	40.26	129.33	34.73
1:1	244.00	47.75	37.00	81.33

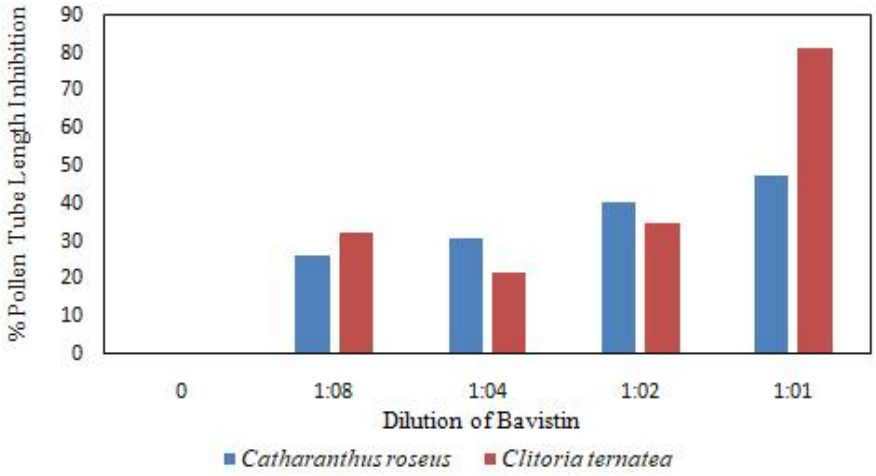

Figure 4. Inhibition of pollen tube elongation by Bavistin

Discussion

In the present investigation Bavistin was found to be inhibitory to both pollen germination and pollen tube elongation in *C. roseus* as well as *C. ternatea*. Several investigators have reported that fungicide treatment caused inhibition of *in vitro* pollen germination and pollen tube growth in almond (Yi et al., 2003; Zarrabi and Imani, 2011), *Brassica campestris* (Pavlík and Jandurová, 2000), *Brassica juncea* (Jain et al., 2000), peach and nectarine (Kargar and Imani, 2021). The inhibitory effect could be due to the interference with nutrient uptake or pollen metabolism by the fungicide (Holb, 2008). An alternative mechanism could be inhibition of formation or fusion of Golgi vesicles or both, processes which are needed for formation and elongation of the pollen tube (Subramanyan et al., 2023). The hydration of pollen grains did not appear to be hampered by the presence of fungicide which was also observed in case of *Brassica campestris* suggesting that the fungicide does not influence the uptake of water. Therefore, the reduction of pollen germinability and delay in germination of pollen of *B. campestris* in response to fungicides have been attributed to retardation of the synthesis of pollen wall compounds or RNA synthesis by the fungicide (Pavlík and Jandurová, 2000).

The population of pollen grains of a flower/plant represent segregated dominant and recessive genotypes since pollen grains are products of meiosis. As some of the pollen grains are able to germinate in presence of the fungicide it may be assumed that some stress tolerant genotypes exist in the plants (Pavlík & Jandurová, 2000). The inhibition of germination of pollen in the present study was evidently concentration dependent. In presence of lower concentrations of Bavistin, pollen germination and pollen tube growth were higher. Thus, it may be possible to determine the minimum inhibitory concentration of the toxic compound for safe use on the plants for control of diseases.

Conclusion

The fungicide Bavistin inhibited *in vitro* pollen germination and pollen tube elongation in *Catharanthus roseus* and *Clitoria ternatea* in a concentration dependent manner. The results clearly point to the cytotoxicity of the fungicide in pollen grains. Therefore, Bavistin should not be sprayed on the plants before fruit set and that too only after investigating the impact of Bavistin on fruit and seed set in plants.

Acknowledgements

The authors express their gratitude to Professor Bijayalaxmi Nanda, Principal, Miranda House, for providing the facilities to conduct this research project under the aegis of DSKC Kothari Centre for Research and Innovation in Science Education, Miranda House. The authors also

thank the laboratory staff members of the Department of Botany, Miranda House, for their support.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Ali Zarrabi, A. and Imani, A. 2011. Effects of fungicides on *in-vitro* pollen germination, tube growth and morphology of almond (*Prunus dulcis*). Afr. J. Agric. Res. 6(25): 5645-5649. DOI: 10.5897/AJAR11.957.
2. Bantawa, P., Roy, O.S., Ghosh, P. and Mondal, T.K. 2009. Effect of Bavistin and Adenine Sulphate on *in vitro* Shoot Multiplication of *Picrorhiza scrophulariiflora* Pennell.: An Endangered Medicinal Plant of Indo-China Himalayan Regions. Plant Tissue Cult. & Biotech. 19(2): 237-245.
3. Bilderback, D.E. 1981. *Impatiens* pollen germination and tube growth as a bioassay for toxic substances. *Environ. Health Perspect.*37:95-103. DOI: 10.1289/ehp.813795.
4. Brewbaker, J.L. and Kwack, B.H. 1964. The calcium ion and substances influencing pollen growth. In: Linskens HF (ed). Pollen Physiology and Fertilization. Amsterdam: Elsevier North Holland; pp. 145-151. DOI:10.1002/J.1537-2197.1963.TB06564.X.
5. Chopra, A., Goyal, S., Singhal, P., Gupta, M., Singh, R., Sharma, A.K. and Sharma, P. 2023. An optimized protocol for seed. Sterilization and shoot regeneration from mature embryo in wheat (*Triticum aestivum*L.) *var*. HD2967. Lett. Appl.NanoBioScience 12(4): 129. https://doi.org/10.33263/LIANBS124.129.
6. Gentile, A.G., Gallagher, K.J. and Santner, Z. 1971. Effect of some formulated insecticides on pollen germination in tomato and Petunia. J. Economic Entomol. 64:916-919. https://doi.org/10.1093/jee/64.4.916.
7. Gentile, A.G., Vaughan, A.W. and Pfeiffer, D.G. 1978. Cucumber pollen germination and tube elongation inhibited or reduced by pesticides and adjuvants. Environ.Entomol. 7: 689-691. https://doi.org/10.1093/ee/7.5.689.
8. Gentile, A.G., Vaughan, A.W., Richman, S.M. and Eaton, A.T. 1973. Corn pollen germination and tube elongation inhibited or reduced by commercial and experimental formulations of pesticides and adjuvants. Environ. Entomol. 2:473-476. https://doi.org/10.1093/ee/2.3.473.
9. Ghurde, M.U., Chokse, G.J. and Malode, S.N. 2021. Effect of fungicide Bavistin on growth and chlorophyll content in *Triticum aestivum* L. International e-Conference on New Horizons and Multidisciplinary Applications in Science and Technology in association with International Journal of Scientific Research in Science and Technology 9(6): 683-688.
10. Holb, I.J. 2008. Influence of pesticide use on flower formation and fertility of some fruit species Int. J. Hortic. Sci. 14(1-2): 103-106.
11. Jain, A., Dhingra, H.R., Mehta, N. and Sangwan, M.S. 2000. Effect of fungicides on *in vitro* pollen germination and tube growth in *Brassica juncea.* Indian Phytopathol. 53(3): 329-330. Retrieved from https://epubs.icar.org.in/index.php/IPPJ/article/view/19333.
12. Kamble, S.I. 2005. Effect of herbicide glyphosate on pollen germination in vitro and pollen tube length of *Hibiscus cannabinus* Linn. Biosci. Biotechnol. Res. Asia3(2): 335-340.

13. Kargar, M.H. and Imani, A. 2011. Effects of fungicides on pollen germination peach and nectarine *in vitro.* Afr. J. Plant Sci.5(11): 643-647.
14. Kumar, D., Sengar, R.S., Yadav, M.K., Pooranchand, Singh, G. and Gupta, S. 2019. Evaluation of sterilant effect on *in-vitro* culture establishment in sugarcane variety Co 0118. Int. J. Curr. Microbiol. App. Sci 8(7):1226-1233. https://doi.org/10.20546/ijcmas.2019.807.146
15. Mehri, H., Mehri-Kamoun, R., Ben Dhiab, A. and El Mahjoub, M. 2006. The effect of bactospeine and three insecticides on olive pollen germination and tube growth. Adv.Hortic. Sci.20(2):140-146.
16. Meshram, R. and Chaturvedi, A. 2017. Effect of insecticide on in vitro pollen germination of *Lycopersicon esculentum* (Mill.) of F1 hybrid variety Laxmi. International Journal of Applied and Natural Sciences, 6:1-10.
17. Nikolov, A., Botiyanski, P., Kehayov, D. and Roichev, V. 2000. Effect of some insecticides on pollen germination of grape varieties Bolgar and Cabernet Sauvignon. *Bulg. J. Agric. Sci*.6:29-31.
18. npic portal- http://npic.orst.edu/ingred/ptype/fungicide.html
19. Padilla, F., Soria, N., Oleas, A., Darwin, R., Manjunatha, B., Kundapur, R.R., Maddela, N.R., and Rajeswari, B. 2017. The effects of pesticides on morphology, viability, and germination of blackberry (*Rubus glaucus* Benth.) and tree tomato (*Solanum betaceum*Cav.) pollen grains. 3 Biotech. 7(3): 154-231. doi: 10.1007/s13205-017-0781-y. Epub2017 Jun 16.
20. PalDat - Palynological Database A- https://www.paldat.org/pub/Catharanthus_roseus/301079.
21. PalDat - Palynological Database B- https://www.paldat.org/pub/Clitoria_ternatea/303537
22. Pavlík, M. and Jandurová, O.M. 2000. Fungicides cytotoxicity expressed in male gametophyte development in *Brassica campestris* after in vitro application of converted field doses. *Environ. Exp. Bot*. 44: 49-58. DOI: 10.1016/s0098-8472(00)00053-8.
23. Preethi, D., Sridhar, T.M. and Naidu, C.V. 2011. Effect of bavistin and silver thiosulphate on *in vitro* plant regeneration of *Stevia rebaudiana.* J. Phytol. 3(5):74-77.
24. Reddy, S.H., Chakravarthi, M., Chandrashekara, K.N. and Naidu, C.V. 2012. Influence of bavistin and silver thiosulphate on *in vitro* regeneration of *Asclepias curassavica*(L.) using nodal explants. Am. J. Plant Sci. 3: 941-946. http://dx.doi.org/10.4236/ajps.2012.37111.
25. Salgare, S.A. 2006. Effect of gramoxone on pollen germination and tube growth of twelve hours stored pollen of apocynaceae and further evidence of a criticism of the hypothesis of Sudhakaran (1967) – a critical review. Plant Arch. 6:389-390.
26. Shivanna, K.R. 2003. Pollen biology and biotechnology special Indian edition (pp. 66-67). New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.
27. Shivanna, K.R. and Rangaswamy, N.S. 1992. Pollen biology: a laboratory manual. Berlin, New York, Paris, Hong Kong and Budapest: Springer-Verlag.
28. Subramanyan, J., Chaudhary, N., Chaudhary, D., Pawar, P. and Kumari, S. 2023. Effects of Indoxacarb and Spinosad on faba bean pollen. Int. J. Innov. Res. in Multidiscip. Field 9(1): 8-15. DOIs:10.2015/IJIRMF/202301002.
29. Sutherland, J.R., Woods, T.A.D. and Miller, G.E. 1984. Effect of selected insecticides and fungicides on germination of Douglas fir and white spruce pollen. Tree Planter's Notes 35:22-24.

30. Tiwari, K.N., Tiwari, V., Singh, J., Singh, B.D. and Ahuja P. 2012. Synergistic effect of trimethoprim and bavistin for micropropagation of *Bacopa monniera*. Biol. Plant. 56 (1):177-180.
31. Yi, W.G., Law, S.E. and Wetzstein, H.Y. 2003. An in vitro study of fungicide effects on pollen germination and tube growth in almond. Hort. Sci. 38(6): 1086-1088. DOI:10.21273/HORTSCI.38.6.1086.
32. Yoon, M.-Y., Cha, B. and Kim, J.-C. 2013. Recent trends in studies on botanical fungicides in agriculture. Plant Pathol. J. 29(1): 1-9. doi: 10.5423/PPJ.RW.05.2012.0072.
33. Zubrod,J.P.,Bundschuh, M., Arts, G., Brühl, C.A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J.J., Rohr, J., Scharmüller, A., Smalling, K., Stehle, S., Schulz, R. and Schäfer, R.B. 2019. Fungicides: An overlooked pesticideclass? Environ. Sci. Technol. 53(7): 3347-3365. https://doi.org/10.1021/acs.est.8b04392.

Access this Article in Online	
Quick Response Code	Website: www.ijarbs.com
	Subject: Plant Biology
DOI:10.22192/ijarbs.2023.10.11.006	

How to cite this article:
Saloni Bahri, Madhu Bajaj, Renuka Agrawal, Somdutta Sinha Roy, Mishthi Khurana and Yamini Sharma. (2023). Effect of the Fungicide Bavistin on *in vitro* Germination of Pollen of *Catharanthus roseus* (L.) G. Don. and *Clitoria ternatea* L. Int. J. Adv. Res. Biol. Sci. 10(11): 49-58.
DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.11.006