INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD
ISSN(O): 2455-0620 [Impact Factor: 7.581]
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value : 86.87
Volume - 9, Issue - 1, January - 2023 Publication Date: 10/01/2023

DOIs:10.2015/IJIRMF/202301002 --:-- Research Paper / Article

Effects of Indoxacarb and Spinosad on faba bean pollen

[1]Janaki Subramanyan, [2]Neetu Chaudhary, [3]Disha Chaudhary, [3]Preeti Pawar, [3]Shivani Kumari
[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 Science II Year Students, Miranda House, University of Delhi, Delhi 110007
E-mail: [1]janaki.subramanyan@mirandahouse.ac.in
[2]neetu.chaudhary@mirandahouse.ac.in

Abstract: *Various agrochemicals need to be used in protecting plants against a variety of pathogens and pests in order to obtain good yields. Chemical as well as biopesticide formulations are available. However, the agrochemicals can be harmful to the crop plant and the harvest, thus affecting humans and the ecosystem as a whole. Therefore, it is necessary to have reliable and simple methods of assaying the toxicity of agrochemicals. The pollen is a sensitive system which has been used to study the effects of xenobiotics. Pollen grains can be germinated in vitro in suitable germination media containing the chemical formulation under test. In the present study the pollen of Vicia faba has been used as a suitable system to study the effects of two commonly used insecticides for pests of pulse crops; namely Indoxacarb, a chemical insecticide, and Spinosad, a biological insecticide. The insecticide was incorporated in the simple pollen germination medium (30 % sucrose) at 1:8, 1:4, 1:2 and 1:1 dilution of the recommended dose. The results show that Indoxacarb and Spinosad inhibit pollen germination at all concentrations tested. Indoxacarb inhibited pollen tube elongation at all concentrations tested whereas Spinosad did not inhibit pollen tube elongation at 1:8 dilution. Importantly, Spinosad was comparatively less inhibitory than Indoxacarb at all concentrations. Being less injurious than chemical formulations, biological pesticides should be popularized among farmers.*

Keywords: *Indoxacarb, Spinosad, pollen germination, pollen tube growth, Vicia faba.*

1. INTRODUCTION:

Several agrochemicals are used in agriculture for plant protection to increase the yield and improve the quality of the harvest. Scheduling insecticide sprays, and studying the impact of the sprays on pollinator behaviour and post-pollination processes are important aspects of pest management programmes [1-3]. Studies are also being conducted to identify microbial consortia for bioremediation of organochlorine insecticides [4]. Pesticide residues have been detected in vegetables and fruits [5], and many pesticides are neurotoxins and need to be used with great caution [6]. Seeds and pollen grains can be used as reliable, inexpensive and convenient systems in toxicological assays [7]. Chemical pesticides are more harmful than biopesticides and, therefore, it is necessary that tests are conducted to screen pesticides for their effect on pollen germination before applying in the fields [8]. In vitro pollen germination and pollen tube growth are easy, rapid, sensitive and suitable methods to study the effects of toxic compounds and monitor pollution [9,10]. Pollen germination and pollen tube growth can be obtained within a few hours and do not require aseptic conditions. Many pollen germination media have been formulated [11]. The inhibition of pollen germination and pollen tube elongation in in vitro pollen cultures in presence of toxic substances indicates toxicity at the cellular level. Pollen grains have been used in studying the effects of fungicides, herbicides, insecticides, pesticides, pollutants and toxic substances [12-23] and in biomonitoring air pollution caused by heavy metals [24]. In the present study the effects of two broad-spectrum insecticides Indoxacarb and Spinosad, which are commonly used to control major pests of pulse crops, have been studied on pollen germination and pollen tube growth of faba bean (*Vicia faba* Linn.).

---

[3]Currently Ms. Disha Chaudhary is pursuing M.Sc. Biotechnology at Savitribai Phule Pune University; Ms. Preeti Pawar M.Sc. Zoology from DAV College, Panjab University, Chandigarh; and Ms. Shivani Kumari Integrated Ph.D. in Biological Sciences from Indian Institute of Science Education and Research, Thiruvananthapuram.

For selecting two commonly used insecticides for pests of pulse crops the latest available list of insecticides/ pesticides registered under section 9 (3) of the Insecticides Act 1968 for use in India (as on 31.10.2019) was consulted. Indoxacarb, a chemical insecticide, and Spinosad, a biological insecticide, were easily available in the local seed market and were chosen as the two insecticides. Indoxacarb is a carbamate insecticide which paralyses insects and insect larvae and kills them in 24 to 60 hours. However, Indoxacarb has a low toxicity and is safe for the natural enemies of insect pests. Spinosad is a mixture of spinosyn A and spinosyn B obtained by the fermentation of the soil bacterium *Saccharopolyspora spinosa*, an actinomycete. The nervous system of insects that consume or come in contact with Spinosad is affected causing their muscles to flex uncontrollably. The insects are paralysed and die within two days.

2. MATERIALS AND METHODS:

Faba bean, fava bean or broad bean (*Vicia faba* Linn.), an annual legume crop, was the experimental material. Sufficient quantity of pollen grains can be collected from the flower buds. The pollen grains germinate easily in a simple germination medium containing only sucrose under ambient room temperature (20-24ºC). All experiments were conducted using fresh pollen collected from buds picked in the morning and likely to open the same day. Sitting-drop pollen cultures were raised on microslides using fresh pollen. The cultures were incubated in Petri dishes lined with moist filter paper at room temperature.

All sitting drop pollen cultures were scored 90 minutes from culture. The culture drop was made homogenous using a needle and a coverslip was lowered. Random non-overlapping microscopic fields were chosen for scoring pollen germination and pollen tube lengths. A pollen grain was considered as germinated only when the length of the pollen tube was equal to or more than the long axis of the pollen grain. The technique of micrometry was used to measure pollen tube lengths. Pollen tubes that had burst at their tips were not measured. All experiments were repeated at least three times and the average values were taken for interpreting the results.

2.1 Effect of different concentrations of sucrose on pollen germination and pollen tube elongation

The pollen grains of *V. faba* were germinated in different concentrations of sucrose (10%, 20% and 30%) solutions in order to determine the best suited sucrose concentration.

2.2 Effects of different concentrations of Indoxacarb or Spinosad on pollen germination and pollen tube elongation

To study the effects of Indoxacarb or Spinosad on pollen germination and pollen tube growth, sitting drop pollen cultures were raised in different concentrations of the commercially available Indoxacarb or Spinosad solutions and controls were maintained. The concentration of the stock solutions was prepared according to the dose recommended for the gram caterpillar *Helicoverpa armigera*, a common pest of pulse crops (25). The recommended dose for Indoxacarb 14.5% SC is 6.5 mL/ 10 L and of Spinosad 45% SC is 3.2 mL/ 10 L (SC stands for suspension concentrate). The stock solutions of Indoxacarb and Spinosad were prepared in 30% sucrose. The stock solutions were suitably diluted using 30% sucrose to obtain 1:1, 1:2, 1:4 and 1:8 dilutions volume/ volume. The data were collected from 90-min old cultures.

3. RESULTS AND DISCUSSION:

3.1 Effects of sucrose:

The pollen grains of *V. faba* are produced as monads; the pollen grains are oblong and each pollen grain has two germ pores, one each at a pole (Figure 1 A). Two pollen morphs were observed: opaque and non-opaque. Both types of pollen grains germinated. Percent pollen germination (Table 1) was highest in 30 % sucrose (78.93 %) and lowest in 10% sucrose (32.38 %). Pollen tube elongation (Table 1) was highest in 30 % sucrose (234.38 µm) and lowest in 10 % sucrose (104.19 µm). The percent pollen germination and pollen tube elongation in 20 % sucrose was intermediate when compared to 10 % and 30 % sucrose. Therefore, 30% sucrose was selected as the germination medium in further experiments.

Table 1: Effect of sucrose concentration on pollen germination and pollen tube elongation of *Vicia faba.*

Medium (Sucrose, %)	Average % pollen germination*	Average pollen tube length (µm)**
10	32.38	104.19
20	49.37	167.59
30	78.93	234.38

* Average of four replicates. The total number of pollen grains scored per replicate was from 26 to 297.
** Average of three replicates based on 120 pollen tube measurements for each medium tested.

3.2 Effects of Indoxacarb:

Indoxacarb inhibited pollen germination and pollen tube elongation in all concentrations tested and the percent inhibition increased as the concentration increased (Figure 1). The inhibition of pollen germination was 40.84 % in 1:8 dilution of Indoxacarb and 90.78 % in 1:1 dilution of Indoxacarb (Table 2). The inhibition of pollen tube elongation was 21.68 % in 1:8 dilution of Indoxacarb and 46.78 % in 1:1 dilution of Indoxacarb (Table 3). However, in 1:1 dilution of Indoxacarb, the number of germinated pollen grains in a sitting drop was few; only 6 to 15. In case of 1:2 dilution of Indoxacarb, out of the 6 drops one drop had only 16 germinated pollen grains whereas in the remaining drops at least 20 germinated pollen grains were available for collection of data. Several pollen grains showed one papilla in 1:1 and 1:2 dilutions of Indoxacarb.

The results clearly show that Indoxacarb inhibited pollen tube formation and that is why several pollen grains showed a single papilla in 1:1 and 1:2 dilutions of Indoxacarb. Such pollen grains would not be able to germinate in the time interval studied (90 minutes from incubation). It is likely that Indoxacarb inhibits the formation of Golgi vesicles or fusion of Golgi vesicles or both, which are required for pollen tube formation and elongation.

Table 2: Effects of Indoxacarb and Spinosad on pollen germination of *Vicia faba.*

Medium	Indoxacarb		Spinosad	
	Average % pollen germination*	% Inhibition of pollen germination	Average % pollen germination**	% Inhibition of pollen germination
30% sucrose	71.45	0	63.96	0
1:8 dilution	42.27	40.84	48.27	24.53
1:4 dilution	35.37	50.50	38.31	40.10
1:2 dilution	14.72	79.40	47.51	25.72
1:1 dilution	6.59	90.78	40.37	36.88

*Average of four replicates. The total number of pollen grains scored per replicate was from 114 to 490.
**Average of six replicates. The total number of pollen grains scored per replicate was from 195 to 872.

Table 3: Effects of Indoxacarb and Spinosad on pollen tube elongation of *Vicia faba.*

Medium	Indoxacarb		Spinosad	
	Average pollen tube length (µm)*	% Inhibition of pollen tube elongation	Average pollen tube length (µm)**	% Inhibition of pollen tube elongation
30% sucrose	149.03	0	110.65	0
1:8 dilution	116.72	21.68	115.24	NA
1:4 dilution	94.87	36.34	96.46	12.82
1:2 dilution	88.80	40.41	89.48	19.13
1:1 dilution	79.32	46.78	80.54	27.21

* Average of three replicates based on 120 pollen tube measurements for each medium tested.
**Average of five replicates based on 200 pollen tube measurements for each medium tested.
NA: Not applicable

3.3 Effects of Spinosad:

Spinosad inhibited pollen germination in all concentrations tested (Table 2, Figure 2). However, in 1:8 and 1:2 dilutions the percent inhibition was nearly the same. Similarly, the percent inhibition in 1:4 and 1:1 dilutions was similar. Therefore, with increase in concentration there was no trend in increase of percent inhibition. At 1:8 dilution Spinosad did not inhibit pollen tube elongation (Table 3). The percent inhibition of pollen tube elongation increased as the concentration of Spinosad was increased from 1:4 to 1:1 dilution. In 1:1 and 1:2 dilutions of Spinosad several pollen grains showed one papilla. However, each sitting drop culture had sufficient germinated pollen grains with intact pollen tube tips.

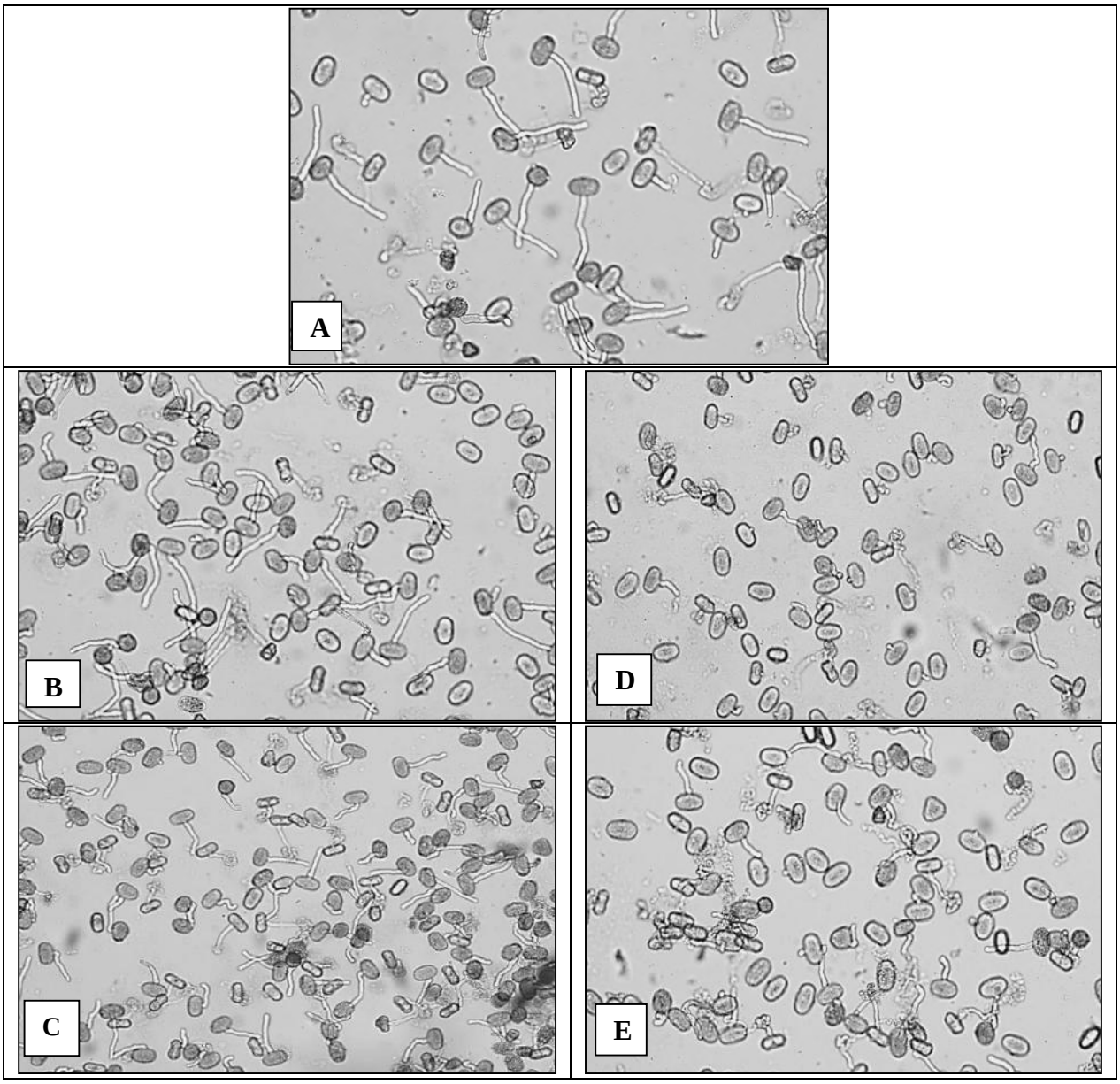

Figure 1: Effects of Indoxacarb on pollen germination and pollen tube elongation of *Vicia faba.* All are representative photographs of 90-min-old cultures. A. Control in 30 % sucrose; B to E: In Indoxacarb at 1:8, 1:4, 1:2 and 1:1 dilution, respectively.

3.4 Comparison of the effects of Indoxacarb and Spinosad:

At 1:4 dilution inhibition of pollen germination caused by Indoxacarb and Spinosad was 50.5% and 40.1%, respectively (Table 2, Figure 3). At 1:1 dilution the inhibition of pollen germination by Indoxacarb was 90.78% whereas the inhibition by Spinosad was only 36.88%. The inhibition of pollen tube elongation by Indoxacarb and Spinosad were 46.78% and 27.21%, respectively, at 1:1 dilution (Table 3; Figure 4). This showed that Spinosad was less inhibitory than Indoxacarb on the pollen system studied. However, Spinosad has been shown to have high toxicity against the pulse beetle [26]. Our results are somewhat similar to those of Mehri et al. [27,28] who studied the effect of Bactospeine, a microbial pesticide obtained from *Bacillus thuringiensis* subsp. *kurstaki* on three cultivars of olive pollen. Addition of Bactospeine to the pollen germination medium did not have any inhibitory effect in a cultivar of olive and increased percent pollen germination in two cultivars of olive; pollen tube elongation was enhanced in the presence of Bactospeine in all the three olive cultivars. Similarly, it was shown by Padilla et al. (2017) that biological pesticides do not affect pollen germination in tree tomato (*Solanum betaceum*) whereas chemical pesticides inhibited pollen germination.

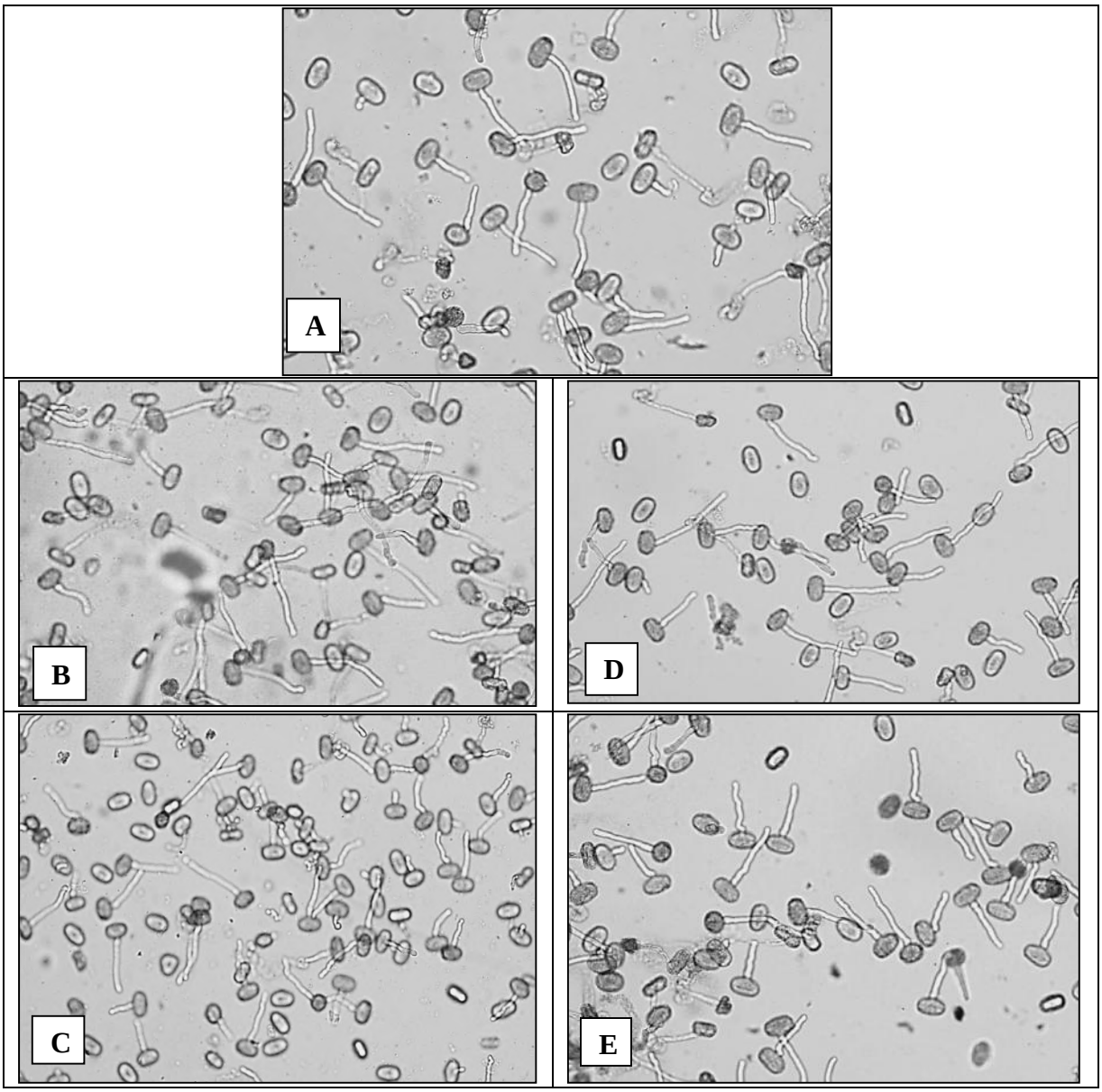

Figure 2: Effects of Spinosad on pollen germination and pollen tube elongation of *Vicia faba*. All are representative photographs of 90-min-old cultures. A. Control in 30 % sucrose; B to E: In Spinosad at 1:8, 1:4, 1:2 and 1:1 dilution, respectively.

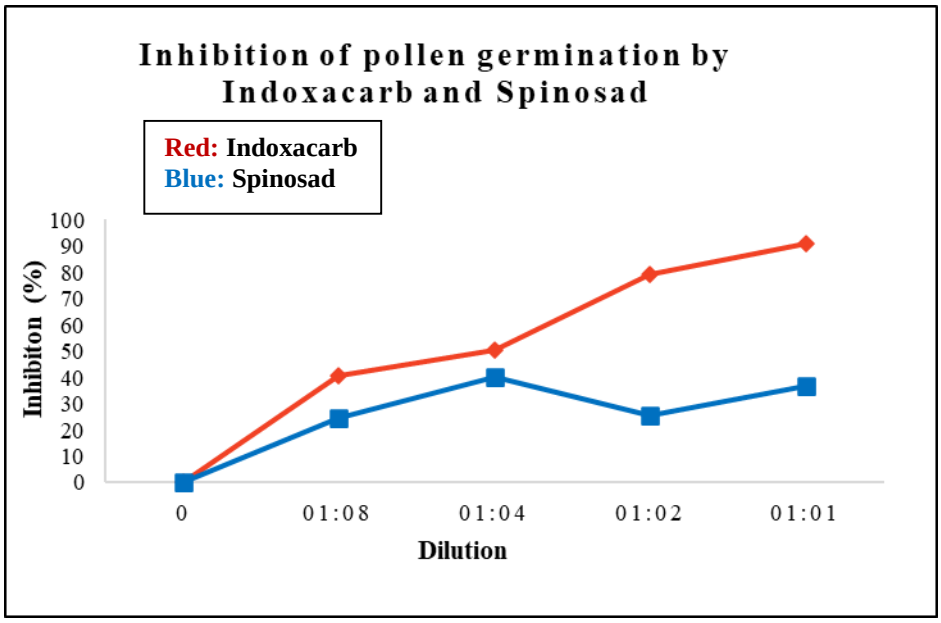

Figure 3: Effects of Indoxacarb and Spinosad on pollen germination.

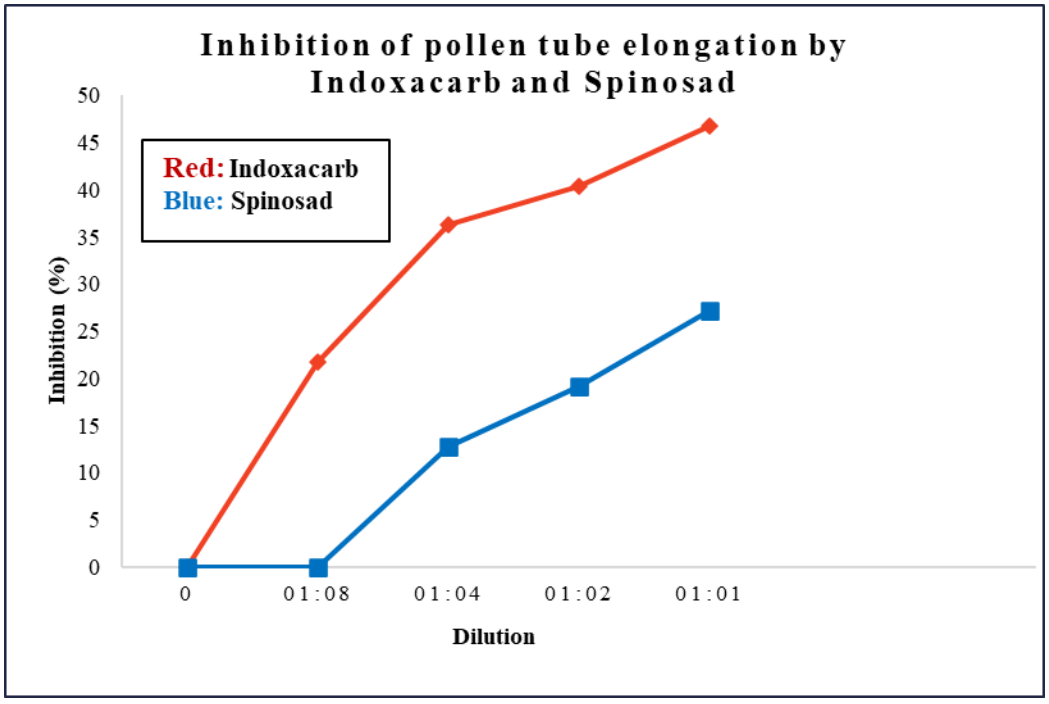

Figure 4: Effects of Indoxacarb and Spinosad on pollen tube elongation.

4. CONCLUSIONS:

Indoxacarb is more inhibitory than Spinosad with reference to pollen germination as well as pollen tube elongation. This can be explained based on the fact that Indoxacarb is a synthetic insecticide whereas Spinosad is a biological insecticide. However, it is also important to note that the insecticides are sprayed when the crop has started to set fruit and will not affect pollination and fertilization. Nevertheless, the inhibitory effects of diluted Indoxacarb and Spinosad show that we must exercise caution while using insecticides in general and synthetic insecticides in particular, especially because most legume crops are used as fodder or mulch. Also, the pesticide residues are likely to be present in the harvested part, be it the vegetable, fruit or seed.

Acknowledgements

We sincerely thank Professor Bijayalaxmi Nanda, Principal, Miranda House, and Professor Sadhna Sharma, Coordinator, DBT Star College Scheme, Miranda House, for encouraging students to undertake short-term projects. We thank Dr. Saloni Bahri, Teacher-in-Charge, Department of Botany, and a Departmental Coordinator, DBT Star College Scheme, Miranda House, for her interest. The laboratory staff of the Department of Botany were very supportive, and we thank them.

Conflict of Interest

The authors declare that there are no conflicts of interest.

REFERENCES:

1. Gillespie S, Long R, Seitz N, and Williams N, (2014). Insecticide use in hybrid onion seed production affects pre- and postpollination processes. *Journal of Economic Entomology, 107*, 29-37.
2. Banik S, Dey P, and Pandit P, (2020). Effect of different insecticides on yield, yield attributes and pollinator behaviour of different pigeon pea varieties. *Legume Research - An International Journal, LR-4387,* 1-4.
3. Viteri DM, and Linares-Ramírez AM, (2022). Timely application of four insecticides to control corn earworm and fall armyworm larvae in sweet corn. *Insects, 13 (3),* 278.
4. Raju SM, and Bidlan R, (2018). Microbial population with potential to simultaneously degrade organochlorine insecticides. *Journal of Experimental Biology and Agricultural Sciences, 6,* 690-700.
5. Bhatnagar A, Dave R, Jindal T, and Virdi JS, (2022). An intensive study on pesticides contamination and its removal in fruits and vegetables collected from Ghaziabad, India. *Journal of Experimental Biology and Agricultural Sciences, 10,* 104-116.
6. Singh N, and Gautam P, (2021). Neurodegenerative diseases: impact of pesticides. *Journal of Experimental Biology and Agricultural Sciences, 9,* 572-579.
7. Kristen U, (1997). Use of higher plants as screens for toxicity assessment. *Toxicology in Vitro, 11* (1-2), 181-191.
8. Padilla F, Soria N, Oleas A, Darwin R, Manjunatha B, Kundapur RR, Maddela NR, 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*, 154-231.
9. Shivanna KR, and Rangaswamy NS, (1992). Pollen biology: a laboratory manual. Berlin, New York, Paris, Hong Kong and Budapest: Springer-Verlag.
10. Shivanna KR, (2003). Pollen biology and biotechnology special Indian edition (pp. 66-67). New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.
11. Jayprakash P, (2018). Pollen germination *in vitro*. http://dx.doi.org/10.5772/intechopen.75360
12. Gentile AG, Gallagher KJ, and Santner Z, (1971). Effect of some formulated insecticides on pollen germination in tomato and Petunia. *Journal of Economic Entomology, 64*, 916-919.
13. Gentile AG, Vaughan AW, Richman SM, and Eaton AT, (1973). Corn pollen germination and tube elongation inhibited or reduced by commercial and experimental formulations of pesticides and adjuvants. *Environmental Entomology, 2*, 473-476.
14. Gentile AG, Vaughan AW, and Pfeiffer DG, (1978). Cucumber pollen germination and tube elongation inhibited or reduced by pesticides and adjuvants. *Environmental Entomology, 7*, 689-691.
15. Bilderback DE, (1981). Impatiens pollen germination and tube growth as a bioassay for toxic substances. *Environmental Health Perspectives, 37*, 95-103.
16. Sutherland JR, Woods TAD, and Miller GE, (1984). Effect of selected insecticides and fungicides on germination of Douglas fir and white spruce pollen. *Tree Planter's Notes, 35*, 22-24.
17. Subramanyan J, and Rangaswamy R, (1985). Effects of caffeine and dichlobenil on a pollen system. *Indian Journal of Experimental Biology, 23*, 281-284.
18. Wolters JHB, and Martens MJM, (1987). Effects of air pollutants on pollen. *The Botanical Review, 53*, 372-414.
19. Rangaswamy NS, and Subramanyan J, 1988. Gossypol affects plant sperms also. *Current Science, 57* (8), 442-444.
20. Subramanyan J, and Rangaswamy R, (1999). Effects of dichlobenil on two tip growth systems: pollen tube and root hair. *National Academy Science Letters, 22*, 113-118.
21. Nikolov A, Botiyanski P, Kehayov D, and Roichev V, (2000). Effect of some insecticides on pollen germination of grape varieties Bolgar and Cabernet Sauvignon. *Bulgarian Journal of Agricultural Science, 6*, 29-31.

22. Salgare SA, (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 Archives, 6*, 389-390.
23. 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.
24. Wang X, Zhang S, Gao Y, Lü W, and Sheng X, (2015). Different heavy metals have various effects on *Picea wilsonii* pollen germination and tube growth. *Plant Signaling & Behavior, 10* (4), e989015.
25. TNAU Agritech Portal Crop Protection; http://agritech.tnau.ac.in/crop_protection/crop_prot_crop_insect-veg_chillies.html
26. Babu SR, Raju SVS, Singh PS, and Sharma KR, (2020). Determination of toxicity of newer insecticide molecules against pulse beetle, *Callosobruchus maculatus* (Fabricius) (Chrysomelidae: Coleoptera) under laboratory conditions. *Journal of Experimental Biology and Agricultural Sciences, 8*, 35-40.
27. Mehri H, Mehri-Kamoun R, Dhiab AB, and El Mahjoub M, (2006). The effect of bactospeine and three insecticides on olive pollen germination and tube growth. *Advances in Horticultural Science, 20*, 140-146.
28. Mehri H, Mehri-Kamoun R, and El Mahjoub M, (2007). In vitro and in vivo evaluation of 3 insecticides and bio-insecticide effects on olive pollen germination and tube growth. *Journal of Agronomy, 6*, 518-525.