International Journal of Applied and Natural Sciences (IJANS)
ISSN (P): 2319-4014; ISSN (E): 2319-4022
Vol. 6, Issue 4, Jun - Jul 2017; 01 - 10

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

EFFECT OF INSECTICIDE ON IN VITRO POLLEN GERMINATION OF *LYCOPERSICON ESCULENTUM* (MILL.) OF F1 HYBRID VARIETY LAXMI

REENA MESHRAM & ALKA CHATURVEDI

Research Scholar, Department of Botany, RTM Nagpur University, Nagpur, Maharashtra, India

ABSTRACT

Pesticides have become an essential part of modern agricultural practices. Pesticides help to reduce pests, which in turn improves various parameters like high yielding, quality of crop, shelf life, etc. In the present investigation, the effect of insecticide Profex super (Profenofos 40% + Cypermethrin 4% EC) was studied on *in vitro* pollen germination of tomato (*Lycopersicon esculentum* Mill.). Insecticide was applied as recommended (30ml/15L), and doubles the recommended dosages (60ml/15L) under field conditions. In recommended dosage, germination goes on decreasing, as the dosage increased as compared to control. In double the recommended dosages, germination again found to decline, as compared to recommended dosages and control.

KEYWORDS: F1 Hybrid, Gibberellic Acid, Improved Germination Medium, In Vitro Pollen Germination, Insecticide, Lycopersicon Esculentum

INTRODUCTION

Pollen plays a very crucial role in plant reproduction, as viability of pollen is a very important factor in the sexual cycle of a plant (Bots and Mariani, 2005). *In vitro* pollen germination is the most commonly used technique in pollen physiology (Heslop-Harrison 1987; Steer and Steer, 1989). It helps in understanding the responses to physical and chemical factors. Pollen germination and pollen tube growth are necessary for fertilization and seed formation in flowering plants. Studies on *in vitro* pollen germination and pollen tube growth are very useful for explaining incompatibility (PFahler et al., 1997; Nurhan, 2003), and also helpful to plant breeders and geneticist for getting better germination in case of field crops. The technique of *in vitro* germination of pollen is extensively used for viability tests, under the general assumption for pollen behavior; pollen that germinates and produces a tube *in vitro* is likely to do so *in vivo*, and to fertilize the egg. In many species, pollen grains germinate and grow on both solid and liquid artificial media. The far and widely used media are modifications of the minimal medium of Brewbaker and Kwack (1964), consisting of 1 to 4 mM H_3BO_3 and 1 to 4 mM $Ca(NO_3)_2$.

To combat productivity with increasing population, number of different pesticides have been used for crop protection and thus for high yield. Several researches have been done to see the detrimental effects of fungicides on pollen germination (Church and Williams, 1977; Redalan 1980; Marcucci *et al*., 1983; Bristow and Windom, 1987; Watters and Sturgeon, 1990; Wetzstein, 1990; He *et al*., 1995; Mussen and Montague, 2004; Holb 2008) and pollen tube growth (Marcucci *et al*., 1983; He *et al*., 1996; Tort *et al*., 2005; Ozturk and Candan, 2010) for commercially important plants. Excessive use of fungicides on pollen under *in vitro* conditions shows a decrease in pollen germination, deformation and cracks in pollen (Lacerda *et al*., 1994; Pavlik and Jandurova, 2000; Holb, 2008). *L. esculentum* is also suffering from a number of diseases, hence the use of intensive pesticides was observed. Similar results were found in tomato flowers, when treated with Mancozeb 3Chlorothalonil 75% WP (3.200 mg/L of a.i.), Mancozeb 80% WP (2.400 mg/L of a.i.),

Mancozeb 48% WP (1.680 mg/L of a.i.) + Metalaxyl 10% WP (350 mg/L of a.i.) and Dibrom 86% EC (1.030 ml/L of a.i.) (Lacerda *et al*., 1994).

"During pollen development, the tapetal layer of the anther wall endows pollen grains with stored products (or their precursors), which are metabolized upon pollen germination and leads to initial tube growth (Stanley and Linskens, 1974; Baker and Baker 1979; Jackson *et al*., 1982; Wetzel and Jensen 1992; Clement *et al*., 1996). Stored phytate is hydrolyzed into phosphate and myoinositol, which are used by the pollen tube for cell wall and membrane synthesis (Jackson and Linskens 1982; Dickenson and Lin 1986). It is assumed that the quantity and quality of these storage products can affect pollen behavior, as measured by percent germination, growth rate of pollen tube, and the capacity to sire seeds (reviewed in Stephenson *et al*., 1994; Delph *et al*., 1997). Thus, any change in the available environment can show changes in the behavior of pollen, i.e. pollen count, viability or percent germination.

It has been observed that, crop yield is directly related to germinability of pollen. The media used for *in vitro* pollen germination varies from species to species and ranges from simple sucrose, boric acid media (Linskens, 1967) to complex media containing polyethylene glycol (Zhang and Croes, 1982; Shivanna *et al*., 1997) and various amino acids (Read *et al*., 1993).

Crop plants are suffering from various bacterial and fungal diseases and insect attack, which results in lower yields. To cope up with such situation, Katara *et al*., (2009) incorporated two pesticides Sycron and Nukem in the germination medium for *Datura metel* L. Then both the pesticides show lethal effects on the species. Similarly, a study was carried out on the effect of Equation Pro (22.5% Famoxadone + 30% Cymoxanil), a fungicide widely used against *Phytopthora infestans* and *Alternaria solani* by Cali (2009) in recommending and double the recommended dosage on pollen viability of tomato, observed under greenhouse condition. He found that at the doses increased, the pollen viability decreased. Cali (2010) studied Aliette, a fungicide having a mixture of WG 800 (80% Fosetyl-Al (Aluminium Tris-o-ethyl phosphonate) to analyze the pollen viability of tomato. He found that the pollen viability decreased with the increasing concentration of doses. It was also reported that, insecticide Profex decreased the pollen count and pollen viability of *Lycopersicon esculentum* as dosage increases (Meshram *et al*., 2015).

It has been observed that, the effect of pesticides, particularly fungicides, were observed by various researchers on pollen germination, but very few focused on insecticide. Similarly, a number of *in vitro* germination media was suggested for different varieties of Tomato, but in present investigation, we did not find results with the same. Hence, present study was designed to see the effect of insecticide (Profex super) on pollen germination of tomato (*Lycopersicon esculentum* Mill.) with modified germination medium for Laxmi F1 hybrid variety.

MATERIAL AND METHODS

Tomato (*Lycopersicon esculentum* Mill.), a member of Solanaceae family was used for the present study. The tomato was selected because, it is used worldwide. Seedlings grown from a variety, Laxmi F1 hybrid (Manufactured by ***nunhems***) in the Post Graduate Teaching Department of Botany, Nagpur. Profex super (Insecticide) (Profenofos 40% + Cypermethrin 4% E.C. Manufactured by Nagarjuna Agrichem Ltd.) was sprayed on plants in flowering condition. Applications were made at the interval of ten days and total five dosages were given. Total eleven dosages, one control and five recommended and five double the recommended dosages were applied under field conditions, i.e. 30ml/15L water as recommended dosage, and 60ml/15L water as double the recommended dosage. Foliar applications were given by sprayer

between 7:00 – 10:30 hours in the morning. After twenty four hours of insecticide spraying, undehisced mature anthers were taken for *in vitro* pollen germination analysis.

In vitro pollen germination study was first done by the method of D. Satish and R. L. Ravikumar (2010), later it was modified.

Pollen Collection and Culture

In vitro pollen germination was done by sitting drop method. Pollen was collected with the help of forceps and needle, anther was dusted on the slide having drop of liquid medium. It was spread evenly in liquid medium. Coverslip was placed and incubated in incubating chamber. Incubating chamber was lined with moist filter paper to maintain the moist condition. Three slides per dosage were prepared and five randomly selected focuses was observed. All observations were taken after 24 hours. The pollen grain was considered germinated, if pollen tube is equal to the diameter of pollen grains.

Pollen Germination Media (PGM)

In this study, the media used by D. Satish and R. L. Ravikumar (2010) was tried. It is consisted of 10% sucrose, 100 mg/L boric acid and 125 µl gibberellic acid. A preliminary investigation was carried in the same media and later it was modified.

For tomato pollen germination, different combinations of sucrose, boric acid and gibberellic acid concentration were used.

Supplementation of Pollen Germination Media with Different Concentrations of Sucrose

Different concentration of sucrose used was 5%, 8%, 10%, 12%, 15%, 16%, 18%, 20%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 32%, 34%, and 36% sucrose.

Supplementation of Pollen Germination Media with Different Concentrations of Boric Acid

Above different sucrose concentrations was supplemented with different concentrations of boric acid as 0.01 gms/100ml, 0.1 gms/100ml.

Supplementation of Pollen Germination Media with Different Concentrations of Gibberellic Acid

Different concentrations of gibberellic acid are 125µl, 275µl, 300µl, 375µl/100ml of solution.

RESULTS

Pollen Germination Media (PGM)

Media suggested for tomato pollen (Pusa variety) by D. Satish and R. L. Ravikumar (2009) was not found suitable for Laxmi F1 hybrid variety. We analyzed different media for the Laxmi variety, by different combinations and concentrations of sucrose, boric acid and gibberellic acid.

Media Supplemented with Different Concentrations of Sucrose

Some of the sucrose percentage which shows germination of pollen grains, but the germinated pollen tubes burst such as 10%, 12%, 15%, 18%, 25%, 30%, and 32%. **In 27% sucrose solution pollen tube was seen.**

Media Supplemented with Different Concentrations of Boric Acid

0.1 gms/100ml and 0.01 gms/100ml of boric acid was found to be effective with different percentage of sucrose like, 10%, 12%, 15%, 18%, 25%, 30%, and 32%. But, pollen tubes burst with these concentrations. With 27% of sucrose, 0.01gms/100ml boric acid concentration was found to be effective as compared to 0.1 gms/100ml of boric acid.

Media Supplemented with Different Concentrations of Gibberellic Acid

375µl gibberellic acid concentration was found to be effective with different concentrations of sucrose and gibberellic acid.

Standardization of Liquid Pollen Germination Media for Laxmi F1 Hybrid Variety

From above trials, the liquid media for **Laxmi F1 Hybrid variety** was standardized as 27% sucrose, 0.01 gms/100ml boric acid and 375 µl gibberellic acid concentrations. The result obtained from these concentrations was better as compared to other different concentrations.

Effect of Insecticide on Pollen Germination

Pollen tube formation is an excellent and simple example of growth and development (Taylor and Hepler, 1997). Therefore, pollen germination and pollen tube growth is an important research tool for morphological, physiological, biotechnological, ecological, evolutional, biochemical and molecular biological studies (Heslop- Harrison, 1992; Ottavio *et al*., 1992; Dane *et al*., 2004). In the present study, the pesticides are sprayed at the flowering stage of plants and observed pollen germination in *L. esculentum*. The data were analyzed with t-test for independent samples.

From table 1 and 2, it is clear that, control shows positive significant difference ($p>0.05$) with PRD-1 to PRD-5 and from PDRD-1 to PDRD-5. Similarly, PRD-1 dose shows positive significant difference ($p<0.05$) with PRD-3, PRD-4 and PRD-5 and from PDRD-1 to PDRD-5 doses. PRD-2 show significant difference with PRD-5, PDRD-3 and PDRD-4. Except these doses, all doses show non-significant difference ($p>0.05$).

DISCUSSIONS

The aim of the present study was to see the effect of Profex, an insecticide on *in vitro* pollen germination of tomato variety Laxmi F1 Hybrid. But at the same time, we standardize the liquid pollen germination media for the same.

"The family Solanaceae is one of the most important families of flowering plants economically, floristically, ethnobotanically and scientifically (Olmstead and Palmer, 1992)". In Angiosperms, the energy required for the various activities like germination, formation of cell wall components and callus formation is provided from the nutriment reserves. These reserved nutriments in pollens play a very important role in the regulation of sucrose concentration during pollen germination. Pollen utilized sugar as an energy source for the synthesis of cell wall material like pectins, cellulose and callose, during pollen tube elongation (Mascarenhas, 1993 and Derksen et al., 1995). It is also reported that, boron added to the medium improves pollen germination and growth in many taxa (Schmucker, 1933; Visser, 1955; Luza and Polito, 1985). Stanley and Loewus (1964) also observed that, boron is instantly involved in pectin synthesis and thus indirectly involved in the development of the pollen tube membrane. Scott (1960) indicated that, boron could exert a protective effect in preventing excessive polymerization of sugars at sites of sugar metabolism. By nature, style provided water, sugar, amino acids to nourish the growing pollen tube. It is also known that, boron is also provided by stigmas and

styles and helps in sugar uptake and has a role in pectin production in the pollen tube (Richards, 1986). Boron deficiency can cause low pollen viability, poor pollen germination and decreased pollen tube growth (Nyomora and Brown, 1997).

Satish *et al*., 2010 used PGM consisted of sucrose 10 percent, boric acid 100mg/L and gibberellic acid 125µl for a Pusan Ruby variety of tomato. But, we found poor results in the same media for Laxmi F1 hybrid variety. We screened a number of different concentrations and combinations of sucrose, boric acid and gibberellic acid. We found excellent results in 27 percent sucrose, 0.01gm/100mL boric acid and 375µl gibberellic acid. In different studies, growth regulators such as yeast, auxins, vitamins, amino acids, etc. are found to be beneficial in pollen germination and pollen tube length. Growth regulators at various concentrations are used for *in vitro* pollen germination media for different plant species (Mascarehas and Canary, 1985). Gibberellins (GAs) are vital endogenous growth regulators of plant. GAs are involved in plant development, including seed germination, trichome development, stem and leaf elongation, flower induction, anther development and fruit and seed development (Pharis and King, 1985; Ross *et al*., 1997; Yamaguchi *et al*., 1998; Kamiya and Garcia-Martinez, 1999; Hedden and Phillips, 2000). GAs are also present in developing pollen after anthesis and a number of studies reported the effect of GA application on pollen tube growth *in vivo* or *in vitro* (Singh et al., 2002). Based on the species and the concentration of GA used, it can promote, inhibit, or have no effect on pollen germination and tube elongation in vitro (Bhandal and Malik, 1979; Viti et al., 1990; Setia et al., 1994).

Gibberellins are reported to use in different *in vitro* pollen germination media (Vasil, 1987). "It is noteworthy that yeast extract was among the earliest growth promoters recommended for pollen (Dorosheko, 1928)". The effect of growth promoters has been suggested by different researchers, they are auxins and vitamins, amino acids, purines and gibberellin (Brewbaker and Kwack, 1963).

In the present work, the percentage of *in vitro* pollen germination in recommending and double the recommended dosage declined as compared to control (Table 1& 2). Similar results were found in the tomato, when treated with some fungicides (Lacerda *et al*., 1994). Similarly, when fungicide was applied on the two cultivars of Peach and Nectarine, then there was decrease in percentage of pollen germination (Kargar and Imani, 2011).

It is common observation that farmers applied pesticides (insecticides, fungicides, herbicides, etc.) extensively on crops; hence, there is an account of the biochemical changes and residual concentration induced in the plants (Pavlik and Jandurova, 2000). In spite of these, our concentrations are as per the recommendation given by the Agrochemical industry. But, we observed decrease in *in vitro* pollen germination when compared to control. There was also an apparent delay in pollen germination and high requirement of growth promoter.

"An *in vivo* test has low sensitivity, as compared to fungicides sprayed on agar, (Marcucci and Filiti, 1984)". Toxic compounds passed through the leaf, and if they penetrate, then they are distributed throughout the plant. Xenobiotics are transported through the xylem and phloem, mostly in apical leaves and upper stems (because of foliar spray), (Jacob and Neumann, 1987). Very small part of it transfers to the anthers, where pollens are produced. These pesticides may be degraded completely or the active compounds metabolized into degraded products, and hence, they are less toxic (Tomlin, 1994). Our observations do not support Tomlin hypothesis. It may be because; we sprayed insecticide after flowering of tomato plants, and pollens are the most sensitive part in the reproductive system. It may also possible that, when foliar spray was given to the tomato plant, it reduces stigma receptivity.

CONCLUSIONS

From the above discussion, it can be concluded that as the dosage of insecticide increased, the pollen germination decreased in case of recommended and double the recommended dosage than control. It will affect the yield. Previously suggested pollen germination media was not found suitable for the Laxmi F1 hybrid tomato variety. The medium developed in this study can be used effectively for *in vitro* pollen selection or interspecific hybridization programs.

Table 1: In Vitro Pollen Germination in Recommended Dosage

S.No.	Dosage of Insecticide	% Germination
1	Control	12.6±1.949
2	PRD-1	10.4±2.96
3	PRD-2	7.2±0.83
4	PRD-3	4.8±1.30
5	PRD-4	4.8±0.83
6	PRD-5	4.4±1.14

PRD- Pofex Recommended Dosage

Table 2: In Vitro Pollen Germination in Double the Recommended Dosage

S.No.	Dosage of Insecticide	% Germination
1	Control	12.6±1.949
2	PDRD-1	5.6±1.14
3	PDRD-2	5±.070
4	PDRD-3	4.8±0.83
5	PDRD-4	5±2.12
6	PDRD-5	5.6±0.89

PDRD-Profex Double the Recommended Dosage

REFERENCES

1. Baker HG, and Baker I. 1979. Starch in angiosperm pollen grains and its evolutionary significance. *Amererican Journal of Botany* **66**: 591–600.
2. Bhandal IS, Malik CP. 1979. Effect of gibberellic acid, (2-chloroethyl) phosphoric acid, actinomycin-D and cycloheximide on the activity and leaching of some hydrolases in pollen suspension cultures of *Crotalaria juncea*. *Physiol. Plant*, **45:** 297-300.
3. Bots M, Mariani C. 2005. Pollen viability in the field. Radboud Universiteit Nijmegen.
4. Brewbaker JL, Kwack BH. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. – *Am. J. Bot*. **50**: 859 – 865.
5. Bristow PR, Windom GE. 1987. Effects of selected fungicides, insecticides, and adjuvants on *in vitro* germination of highbush blueberry pollen. *Plant Dis*., 71: 326-328. cada. *Pesq. agropec. bras. Brasilia* **29**: 1651-1656.
6. Cali I, Candan F. 2010.The effect of activator application on the anatomy, morphology, and viability of *Lycopersicon esculentum* Mill. pollen. *Turk J Biol*. **34**: 281-286.
7. Cali I. 2009. Cytogenetic effects of fungicide applications on meiosis of tomato (*Lycopersicon esculentum* Mill.). *Turk J Biol.* **33**: 205-209.

8. Church RM, Williams RR. 1977. Toxicity to apple pollens of several fungicides, as demonstrated by in vivo and *in vitro* techniques. *J. Hort. Sci*. **52**: 429-436.

9. Clement CM, Burrus JC, Audran. 1996. Floral organ growth and carbohydrate content during pollen development in *Lilium*. *American Journal of Botany* **83**: 459–469.

10. D Satish, Ravikumar RL. 2010. Standardization of *in vitro* pollen germination media in selected varieties of cotton and tomato Karnataka *J. Agric. Sci*., **23** (2): (317-319).

11. Delph, LF, Johannsson MH. Stephenson AG. 1997. How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology* **78**: 1632–1639.

12. Derksen J, Rutten T, Amstel van, de Win TA, Doris F, Steer M. 1995. Regulation of pollen tube growth. *Acta Bot. Neerl*. **44**: 93 - 113.

13. Dickenson DB, Lin JJ. 1986. Phytases of germinating lily pollen. In Mulcahy DL, Mulcahy GB, Ottaviano E. eds. Biotechnology and ecology of pollen, 357–362. *Springer*, New York, New York, USA.

14. Doroshenko AV. 1928. Physiology of pollen. Bull. *Appl. Bot. Plant Breed*. **18**: 217-344.

15. Hedden P, Phillips AL. 2000. Manipulation of hormone biosynthetic genes in transgenic plants. *Curr. Opin. Biotechnol*. **11:** 130-137.

16. Heslop-Harrison Y and Heslop-Harrison J. 1992 Germination of monocolpate angiosperm pollen: Evolution of the actin cytoskeleton and wall during hydration, activation and tube emergence. *Ann Bot*. **69**: 385-394.

17. Holb IJ 2008. Influence of pesticide use on flower formation and fertility of some fruit species *Int. J. Hortic. Sci*., **14**(1-2): 103-106.

18. Jackson JF, Linskens HF. 1982. Phytic acid in Petunia hybrid pollen is hydrolized during germination by a phytase. *Acta Botanica Neerlandica* **31**: 441–447.

19. Jacob F. Neumann S. 1987. Chapter 1. Principles of uptake and systemic transport of fungicides within the plant. In: Lyr H, Fisher G. ed. Modern Selective Fungicides — Properties, Applications, Mechanisms of Action. *Press Verlag, Jena*, pp. 13–29.

20. Kamiya Y, Garcia-Martinez JL. 1999. Regulation of gibberellin biosynthesis by light. *Curr. Opin. Plant Biol.* **2:** 398-403.

21. Kargar MH, Imani A. 2011 Effects of fungicides on pollen germination peach and nectarine *in vitro*. *African Journal of Plant Science*, **5(11)**, pp. 643-647.

22. Katara M, Mankad A. 2009. Effect of insecticides on pollen germination and pollen tube growth of Datura metel L. *Plant International Research Journal*, **3**: 10-11.

23. Lacerda CA, Lima JOG, Almeida EC, Oliveria LM. 1994. Pesticides *in vitro* interference in the germination and in the tube pollinic germination and elongation in the tomato plant cultivar. *Agropec. Bras. Santa Cruz cada. Pesq. Agropec. bras., Brasilia*, **29**: 1651-1656.

24. Linskens HF. 1967. Pollen. *Handbuch der pfanzenphysiologie* **XVIII**, 368-406.

25. Luza JG. Polito VS. 1985. *In vitro* germination and storage of English walnut pollen. *Sci. Hortic*., **27**: 303-316.

26. Marcucci MC, Filiti N. 1984. Germination of pear and apple pollen as influenced by fungicides. *Gartenbauwiss ecschaft*. **49**: 28-32.

27. Marcucci MC, Fiorentino M, Cesari A, Fiaccadori R. 1983. The influence of fungicides on the functioning of apple and pear pollen. In: Pollen: biology and implications for plant breeding. (D.L. Mulcahy and E. Ottaviano Eds.). *Elsevier, Amsterdam*, pp. 73-80.

28. Mascarenhas JP. 1993. Molecular mechanisms of pollen tube growth and differentiation. *Plant Cell*, **5**: 1303 – 1314.

29. Mascarenhas JP. Canary D. 1985. Pollen, symbionts and symbiotic-induced structures. *Encycl. Plant Physiol., New. Ser*., **11**: 579-598.

30. Meshram RS, Chaturvedi A. 2015 Effect of insecticide application on the pollen production and pollen viability of hybrid Tomato (Lycopersicon esculentum Mill.). *International Journal of Institutional Pharmacy and Life Sciences*, **5(3)**, 103-110.

31. Nyomora AMS, Brown PH. 1997. Fall foliar-applied boron increases tissue boron concentration and nut set of almond. *Journal of the American Society for Horticultural Science* **122(3)**, 405-410.

32. Olmstead, RG, Sweere JA, Spangler RE, Bohs L, Palmer J. 1999. Phylogeny and provisional classification of the Solanaceae based on chloroplast Dna. In: ed. Nee M, Symon DE, Lester RN, Jessop JP, Solanaceae IV. Royal Botanic Gardens, Kew, Richmond, Surrey, pp. 111-137.

33. Ottavio E, Mulahy D, Sari Goria M, Mulahy GB. 1992. Angiosperm Pollen and Ovules, Springer-Veriag.

34. Ozturk I, Candant F. 2010.The effect of activator application on the anatomy, morphology, and viability of Lycopersicon esculentum Mill. *Pollen. Turk. J. Biol*. **34**: 281-286.

35. Pavlik M, Jandurova OM. 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.

36. Pharis RP, King RW. 1985. Gibberellins and reproductive development in seed plants. *Annu. Rev. Plant Physiol.* **36:** 517-568.

37. Read SM, Clarke A, Bacic A. 1993. Stimulation of growth of cultured *Nicotiana tabacum* W-38 pollen tubes by polyethylene glycol and Cu (II) salts. *Protoplasma* 177, 1-14.

38. Redalen G. 1980. Effect of fungicides on pollen germination and fruit set in raspberries.*Gartenbauwiss enschsaft*, **45**: 248-251.

39. Richards AJ. 1986. Plant Breeding Systems. George Allen Unwin, London, England.

40. Ross JJ, Murfet IC, Reid JB. 1997. Gibberellin mutants. *Physiol. Plant*, **100:** 550-560.

41. Schmucker, TH. 1933. Zur Blutenbiologie tropischer Nymphaea-Arten. II. Bor ale entscheidences Factor. *Planta*, **18**: 641-650.

42. Scott EG. 1960. Effect of supra optimal boron levels on respiration and carbohydrate metabolism of *Helianthus annuus*. *Plant Physiology* **35:** 653.

43. Setia N, Setia RC, Chabra N. 1994. Interactive effects of growth hormones and calcium antagonists on germination and tube elongation of groundnut pollen. *Plant Cell Incompatibility Newslett*., **26:** 70-80.

44. Shivanna KR, Saxena NP, Seetharama N. 1997. An improvised medium for *in vitro* pollen tube growth of chickpea. *International Chickpea and Pigeonpea Newsletter* **4**, 28-29.

45. Singh DP, Jermakow AM, Swain SM. 2002. Gibberellins are required for seed development and pollen tube growth in *Arabidopsis*. *Plant Cell*, **14:** 3133-3147.

46. Stanley RG, Loewus FA. 1964. Boron and myo-inositol in pollen pectin biosynthesis. In: Pollen Physioilogy and Fertilisation. Linkens HF (Edition). *North-Holland Publishing Company, Amsterdam* 128-139.

47. Stanley RG, Linskens HF. 1974. Pollen: biology, biochemistry, management. *Springer, Berlin, Germany.*

48. Stephenson AG, Erickson CW, Lau TC, Quesada MR, Winsor JA. 1994. Effects of growing conditions on the male gametophyte. In Stephenson AG, Kao TH. ed. Pollen–pistil interactions and pollen tube growth. *Current Topics in Plant Physiology* **12**: 220–229.

49. Taylor LP, Hepler PK. 1997. Pollen germination and tube growth. *Ann Rev of Plant Physiol and Plant Mol Biol* **48:** 461-491.

50. Tomlin C. 1994. The Pesticide Manual. Incorporating the Agrochemicals Handbook. The British Crop Protection Council, Surrey and The Royal Society of Chemistry, *Cambridge*, p. 1341.

51. Vasil IK. 1960. Studies on pollen germination of certain Cucurbitaceae. *Amer J Bot*. **47** (4): 239-248.

52. Visser T. 1955. Germination and storage of pollen. *Meded. Landbouwhogesch. Wageningen*, **55**: 1-68.

53. Viti R, Bartolini S, Vitagliano C. 1990. Growth regulators on pollen germination in olive. *Acta Hort*. **286:** 227-230.

54. Watters BS, Sturgeon SR. 1990. The toxicity of some foliar nutrients and fungicides to apple pollen cv. Golden Delicious.Tests of agrochemicals and cultivars. *Ann. Appl. Biol*., **116**(Suppl.): 70-71.

55. Wetzel CLR, Jensen WA. 1992. Studies of pollen maturation in cotton: the storage reserve accumulation phase. *Sexual Plant Reproduction* **5**: 117–127.

56. Wetzstein HY. 1990. Stigmatic surface degeneration and inhibition of pollen germination with selected pesticidal sprays during receptivity in pecan. *J. Am. Soc. Hort. Sci*., **115**: 656-661.

57. Yamaguchi S, Smith MW, Brown RG, Kamiya Y, Sun TP. 1998. Phytochrome regulation and differential expression of gibberellin 3β-hydroxylase genes in germinating *Arabidopsis* seeds. *Plant Cell*, **10:** 2115-2126.

58. Zhang HQ, Croes AF. 1982. A new medium for pollen germination *in vitro*. *Acta Botanica Neerlandica* **31**: 113-119.

APPENDIX

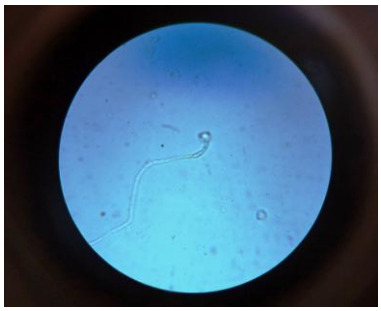

Figure 1: Pollen Germination in Modified Medium