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Biochemical, biogenic elements, and antimicrobial features contributing to reproductive success of pollen in sunflower (*Helianthus annuus* L)

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ABSTRACT

Pollen grains are produced by plants during sexual reproduction and their viability is critical for reproductive success of flowering plants. Pollen is species specific and resistant to chemical and physical attacks provided by environment. Present investigation undertakes histological and qualitative localization of biomolecules, which are required for successful pollination and germination of Helianthus annuus L pollen. These molecules provide structural and functional stability, immunity and ecological specificity to pollen. These biomolecules (lipids, carbohydrates, proteins, polyphenols) also act as signaling molecules on successful adhesion and hydration of pollen. The antimicrobial activity of the pollen has been found against Escherichia coli (gram negative) and Staphyloccocus spp. (gram positive) and has been compared with enrofloxacin (antibiotic drug). Biogenic elements namely, potassium, calcium and boron also have a critical role in the functioning of pollen. Localization and quantification of these biogenic elements provide an insight into their physiochemical importance in pollen.

Keywords: *Asteraceae, polyphenols, pollen grains, signaling molecules*

Introduction

Reproduction is a critical event in the life cycle of flowering plants, which leads to the formation of seeds, and continuation of species on Earth. Programic phase is the critical period in plants which is still a mystery for many scientists working in the field of reproductive biology of plants. The programic phase initiates with the development of the reproductive gametes, pollination and culminates with fertilization of the gametes. Angiosperms possess distinct reproductive traits, including highly reduced male and female gametophytes, prezygotic mate recognition systems, internalized pollen tube growth, and closed carpels. The degree of gametophyte reduction varies among families, reflecting evolutionary adaptations. Pollen grains, the highly reduced male gametophytes of flowering plants, function to deliver viable male gametes to the female gametophyte. Morphological and physiological traits of pollen are species- and family-specific. They are typically released in a desiccated, metabolically inactive state, but upon contact with the stigma surface, rapidly hydrate, germinate, and produce pollen tubes for gamete transfer. The biochemical composition of pollen facilitates these processes. Studies report that pollen contains polyphenols, carotenoids, proteins, lipids, vitamins, and biogenic elements (1,2,3). These compounds confer antioxidant (4), antimicrobial, antifungal (5), and anticancer (6) properties. Polyphenols, including phenolic acids, flavonoids, lignans, and stilbenes, contribute to aroma, color, and bitterness, aiding pollinator attraction, and provide defense against pathogens and UV radiation (7). Previous work has identified polyphenols and flavonoids in sunflower inflorescences (8). Flavonoids, such as quercetin, also protect against ROS-induced DNA damage (9) and exhibit anti-inflammatory, antioxidant, and lipid peroxidation inhibitory effects (10). Carotenoids, along with quercetin, accumulate in chromoplasts, imparting yellow to orange coloration (3,11).

Pollen proteins, located in the exine, intine, or pollen coat, may function as hydrolytic enzymes, recognition molecules, or signaling mediators in plant–pollinator interactions (12). Lipids—phospholipids, fatty acids, and waxes—are critical structural components of the pollen wall. Genes involved in long-chain fatty acid (LCFA) biosynthesis, such as ECERIFERUM (CER1, CER3) and Long-Chain Acyl-CoA Synthetases (LACS), are essential for normal pollen coat formation, with mutations resulting in structural abnormalities (2). Neutral lipids stored in lipid droplets are also vital; defects in triacylglycerol (TAG) synthesis enzymes, such as PDAT1 and DGAT1, cause microspore abortion in Arabidopsis (Herandez et al., 2020). Beyond plant reproduction, pollen constituents are nutritionally important for bees, influencing larval development, immune function, reproduction, and longevity (13,14). Pollen biomolecules also have reported antioxidant, anti-inflammatory, and antinociceptive activities. For example, Scaptotrigona affinis postica pollen extract inhibits cyclooxygenase (COX), a key enzyme in prostanoid biosynthesis (15). Collectively, pollen chemistry underpins its ecological, nutritional, and pharmaceutical significance.

Present investigations have been carried out to examine the morphology and biochemical constituents of pollen in Helianthus annuus var. KBSH, with help of light microscopy and scanning electron microscope and confocal microscopy. The biogenic elements play an important role in pollen grains, and they have been quantified by flame photometer and localized by energy dispersive X-ray analysis (EDAX). An attempt has been made to correlate the biochemical constituents with antimicrobial activity of pollen, examining their potential antimicrobial activity against gram positive (*Stapylococcus* spp.) and gram negative (*Escherichia coli*) bacteria.

Materials and Methods

Sunflower seeds (*Helianthus annuus* var. KBSH) were sourced from the University of Agricultural Sciences, Bangalore (India). After thorough washing, the seeds were immersed in distilled water for 4 hours and then planted in the Multanimal Modi College Garden in Modinagar (India). The plants were cultivated until they reached the reproductive stage. Upon the formation of flowers, they were carefully excised during anthesis at stage 5.3, as per the classification by Scheiter and Miller (16). At this stage, approximately 30% of the head area was in the flowering stage. The florets in the staminate stage and their respective pollen grains were meticulously collected for subsequent experiments.

Localization of biogenic elements

For further analysis, the florets in the staminate stage were fixed using a solution comprising 2.5% glutaraldehyde and 2% paraformaldehyde, prepared in 0.2M sodium cacodylate buffer (pH 7.4), for a duration of 4-5 hours at 25 °C. Subsequently, they were transferred to a buffer solution. The fixed tissue samples underwent a dehydration process following the method outlined by Feder and O'Brien (17). In brief, the fixed tissues were sequentially treated with methoxy ethanol, ethanol, and n-propanol for 24 hours each, followed by n-butanol for 12 hours. Glycol methacrylate (2-hydroxyethyl methacrylate) served as the embedding medium, and sections measuring 2-3 µm were obtained using a rotary microtome. Total carbohydrates were localized by Periodic Acid Schiff's (PAS) reagent, according to Feder and O'Brien (16). Sections were treated with 1 % aqueous dimedone for 24 h at room temperature to block the free aldehyde groups and then treated with 1 % (v/v) periodic acid for 30 min. They were then treated with PAS reagent in dark for 30 min and then passed through three successive changes of 0.5 % (v/v) sodium metabisulfite to remove excess stain. Proteins were localized by staining with 0.2% Coomassie Brilliant Blue R-250 dissolved in a mixture of methanol: acetic acid: water (5:7:88) for 5 min. Lipids were localized by staining with 0.3% Sudan Black B (18).

Localization and quantification of antioxidants

Pollen grains were harvested from the staminate stage of floret development. One gram of the material was extracted in 80% methanol at 50 ◦ C for 12h,in soxhlet. The extract was filtered, decolorized and deffated with petroleum ether. The extract was reduced and concentrated to 3ml by rotary vacuum evaporator. Reversed phase C18 column (Thermoscientific, 5µm, 4.6x250mm) at 30 ◦ C was used with diode array detector set at 190 to 600 nm. Mobile phase was formed of 0.1% formic acid & water (solvent A) and acetonitrile containing 0.1% formic acid (Solvent B). 20µL of the solvent extract was injected (passed through the microfilter), and elution was performed at 1.2 ml/min and detected at 280 nm. Standard (Quercetin) at various concentrations was injected into the HPLC system and calibration curve was established. Concentration of the compound was calculated from peak area according to the calibration curves, and the amount of quercetin was expressed as ppm/ml. Antioxidants, were localized by DPBA (diphenylboricacid 2-aminoethylester). Fixed anthers were stained with 0.1% DPBA in 0.1M potassium phosphate buffer (pH 6.8,1% NaCl). Fluorescence was observed following UV excitation at 405 nm and emission of 580 nm using Diode laser and pin hole of 600 µm (Leica, USA).

Estimation and localization of Biogenic Elements

Potassium (K), calcium (Ca), and boron (B) were quantified using flame photometry (20,21). Briefly, 1 g of pollen was digested overnight at 25 °C in 6 ml nitric acid, followed by heating at 90–100 °C until near-complete acid removal. Further digestion was carried out with 2 ml perchloric acid at 150 °C. Digests were diluted to 25 ml with distilled water, and K and Ca were estimated against standard curves prepared from $CaCO_3$ (2–300 mg L^{-1}) and KCl (2–100 mg L^{-1}) stock solutions.

Boron was estimated following the Azomethine-H colorimetric method (22). One gram of pollen was ashed at 500 °C for 4 h in a muffle furnace, treated with 2.5 ml 2 M HCl, allowed to stand for 15 min, and diluted with 10 ml distilled water before filtration (Whatman No. 40). To 1 ml of filtrate, 2 ml buffer-masking solution (31.25 g ammonium acetate, 1.875 g EDTA disodium salt in 50 ml deionized water, with 15.625 ml glacial acetic acid added slowly) was added, followed by 2 ml Azomethine-H reagent (0.112 g in 25 ml 1% ascorbic acid). After 30 min, absorbance was recorded at 420 nm, and B content was calculated using a standard curve (1–6 mg L^{-1}) prepared from H_3BO_3.

Calcium and potassium distribution in freshly harvested and germinating pollen grains was examined by scanning electron microscopy (SEM) with energy-dispersive X-ray analysis (EDAX), following Zuberi & Dickinson (1985) and Zhao et al. (2004). Samples were gold-coated using a sputter coater (Emitech; Oxford Instruments), mounted on stubs, and analyzed with a Zeiss SEM fitted with an X-ray microanalysis system.

Antimicrobial Assay

Antimicrobial activity of pollen extract was assessed against *Escherichia coli* (Gram-negative) and *Staphylococcus* spp. (Gram-positive) isolates obtained from milk samples (25). Sensitivity testing followed the disk diffusion method (26). Mueller–Hinton agar (MHA) plates were inoculated with 100 µl of bacterial culture and spread evenly. Sterile disks were coated with 10 µl pollen extract and placed on the plates; enrofloxacin (10 µg) served as a positive control. Duplicate plates were incubated at 37 °C for 24 h, and antimicrobial activity was evaluated based on the diameter of inhibition zones.

Results

The inflorescence of sunflower is a capitulum, a compound inflorescence, consisting of disc and ray florets. The ray florets are yellow, strap shaped, zygomorphic, sterile and present on the margins of the head, whereas the disc florets are arranged in a spiral manner in the interior of head. The disc florets are hermaphrodite and mature centripetally in whorls and, therefore, can be seen in different maturation stages. Pollen grains are released in the staminate stage of floret development (Figure 1A). A floret at staminate stage consists of an inferior ovary, pappus (modified calyx), tubular fused corolla, syngenesious anther tube enclosing stigma (Figure 1B). The five stamens consist of fused anthers with free filaments surrounding the style. The florets exhibit protandry and, as the stigma matures, it grows through the anther tube and pushes the pollen out. The anthers are about 10-12 mm in length and are blackish in color. The tip of the anthers has a sharp concave tip with glandular and non-glandular trichomes. The pollen aggregate on these tips when pushed out of the anthers. Transverse section of mature anthers reveal polysporangiate condition with five pollen sacs and outer epidermal layer

becomes thin, shrinks and loses connection with internal tissue (Figure 1B). The pollen grains of sunflower are echinate in ornamentation and sometimes circular in polar view and prolate in equatorial view (Figure 1C). Mature pollen grains are triporate and angulapertuarate. Pollen grains contain an inner pectocellulosic layer, intine and outer exine. Exine is made up sporopollenin and in mature pollen is thick and has thin spine like projections. Pollen coat fills the space between and cavities that are present in the ornamental exine. Pollen coat is extracelluar matrix derived from the tapetum of the anther. Insoluble carbohydrates were detected by PAS staining (Figure 1D). Cellulose can be detected on the intine of the pollen grains. Some carbohydrates may also be present as cytoplasmic carbohydrates which may provide nourishment to the pollen. Carbohydrates may also be present in the exine representing the pollen coat. Proteins localized by Commaisse staining, are present in cytoplasm and intine of the mature pollen grains (Figure 1E). Lipids localized by Sundan Black B, are concentrated on the exine layer, representing the pollen coat and the intine layer (Figure 1E). Some lipid deposition is also evident in the cytoplasm of the pollen representing lipid droplets.

Antimicrobial activity in pollen

The antimicrobial activity of the pollen extract was assessed against both gram-positive Staphylococcus spp and gram-negative Escherichia coli using the disk diffusion assay. Enrofloxacin, the standard drug, was employed as a positive control in the study. Surprisingly, the inhibitory effect of the pollen extract was found to surpass that of the standard drug, enrofloxacin. In the case of Escherichia coli, the inhibitory zones around the disks impregnated with the pollen extract (Figure 1 L) were notably larger, ranging from a maximum of 25mm to a minimum of 23mm, compared to enrofloxacin, which exhibited a maximum of 16mm and a minimum of 13mm (Figure 2A,B). Similarly, when assessing the inhibitory effect against Staphylococcus, the clear zones around the disks impregnated with the pollen extract (Figure 2C,D) were significantly greater, ranging from a maximum of 26mm to a minimum of 24mm, compared to enrofloxacin, with a maximum of 21mm and a minimum of 18mm (Figure 1). The antibiotic activities demonstrated that the pollen extract has the potential to serve as a more effective component of antibiotic drugs, as illustrated in Figure 2E.

Antioxidants are present in pollen

Polyphenols are natural products which have antioxidant properties. Polyphenols include phenolic acids, lignans, flavonoids and stilbenes. HPLC analysis of pollen extracts reveal the presence of quercetin, on the basis of retention time and ultraviolet spectrum and standard reference of quercetin (Figure 3 A,B). The reference peak of quercetin was used to calculate the amount of quercetin in the pollen based on its proportion of areas (Figure 3 C,D-inset). Earlier studies conducted by the author have revealed the presence of phenolics, flavonoids and anthocyanin in pollen. Among the flavonoids, quercetin has been detected by HPLC and by confocal microscopy as well. The quercetin content in pollen was found to be 0.8 mg/gm (Fig. 3D inset). Flavonoids are present on the outer side of pollen, mainly on exine and pollen coat as compared with control (Figure 3 E,F).

Biogenic elements in pollen

Biogenic elements are important component of pollen, which provide it viability and are also important for the various biochemical process occurring in pollen. Pollen when shed are subsperoidal in shape, with polar axis of about 17µm and equatorial axis of about 14 µm (Figure 4A). Potassium, calcium and boron were investigated in pollen. It was found out that a maximum concentration of potassium, 1150 mg g-1 dw, which was about 90% as compared to the content other two biogenic elements, potassium and boron in pollen (Figure 4B). Energy dispersive X ray analysis (EDAX) spectra coupled with scanning electron microscope indicate the presence of potassium peak in the mid region of the furrow (Figure 4 C,E). Calcium accumulation in pollen was about, 100 mg.g -1dw which accounted for about 9% in composition, as compared to potassium and boron concent in pollen. X ray analysis (EDAX) spectra coupled with scanning electron microscope indicate the presence of calcium peak in the mid region of the colpus area (Figure 4 D,F). This area corresponds to the intine region from where the pollen tube emerges upon hydration. Boron concentration was found to be 4 mg g-1dw (Figure 4B), which is less than 1%, as compared to content of calcium and potassium in pollen. Boron accumulates as a micronutrient in the cytoplasm of pollen. As soon as the pollen is hydrated, pollen tube elongates, and shows a characteristic tip growth (Figure 4H). Polarized growth of the pollen tube requires a number of signaling molecules, or biomolecules which aid in the growth and nutrition of the pollen tube. As can be observed (Figure 4 G,I) calcium and potassium accumulate in the pollen tube and form a gradient inside the pollen tube.

Discussion

Anthers exhibit synchronized development

During the course of evolution, sunflower (*Helianthus annuus*) has undergone various morphological, anatomical and biochemical changes to ensure pollination through entomophily. The capitulum of sunflower has disc florets which mature in a concentric manner. The disc florets form a target pattern which is visible in UV light. Protandry is regulated by photoperiodicity, in sunflower which induces different speed of elongation of style and filament of anthers (27). Auxins also involved in light induced elongation of cells. An interplay of light and hormones (auxins and gibbbrellins) is perceived by corolla which results in the proper development of style and filaments (28). At maturity, the outer anther wall, (epidermis) encloses the pollen grains. Sunflower has been reported to have peripalsmodial taptetum which disintegrate and forms the pollen coat on maturity of pollen. Exine is further formed of inner nexine and outer sexine, which collectively form the ornamentation of the pollen. The sexine is further differentiated into tectal element/spines/sculptural elements, tectum and columella (2). Exine is formed of sporopollenin which consists of phenylpropanoids, phenolics, proteins and fatty acid derivatives (30). Proteins that originate from the tapetum attach on the cavities of exine and act as allergens and recognition molecules for interspecific compatibility. Several reports have suggested that phenolic and aromatic compounds are present on/in the exine. Sporopollenin provides physical strength and resistance to non-oxidative chemical and biological degradation. Present investigations reveal the presence of polyphenols on the exine of pollen (Hernandez et al. 2020). These polyphenols (flavonoids, flavonols, anthocyanins) not only protect the pollen against UV, but also act as semiotic

signals for the pollinators. The pollen and nectar are produced in the staminate stage of floret which is collected as a reward by the honey bees . Antioxidants like quercetin and Nitric oxide (NO) scavenge the reactive oxygen species presnt on the receptive stigmatic papillae cells and initiate ROS scavenging system and signaling cascades (31). The antioxidant activity of quercetin is much higher and has been used in food to reduce disease and promote health.

Biogenic elements present in the furrows and apertural regions have a role in harmomegathy

Pollen apertures are the regions where exine is reduced significantly. This allows shrinkage of pollen (during desiccation) and swelling (on uptake of water) from the stigma. During the process of dehydration of pollen in the anthers, the membrane of the aperture site folds inwards, so that the apertural edges touches each other and a furrow is created. These apertures and the pollen exine play a role in harmomegathy, allowing retraction (bending) of wall during dehydration or extending during hydration. The presence of potassium ions in the furrows in present investigation further support their role in harmomegathy, and accommodate volume-change (32). Earlier studies in maize have also revealed high concentration of potassium in pollen(3). Present investigation show the presence of potassium in the furrow aperture area of pollen grains where it is likely to function as a turgor regulator, resulting in the swelling of pollen. Potassium is required for pollen germination and tube growth (33). The pollen grain on hydration takes up potassium and calcium from the stigma. In *Arabidopsis*, pollen tubes swell in the presence of high external potassium concentration (34). Pollen tube growth requires a high potassium influx through potassium channels. Both stretch-activated and spontaneous potassium channels have been identified in the pollen grains (35). Potassium influx as well as the consequent water influx, are required for the maintenance of turgor pressure in the pollen tube.

Calcium, potassium and boron help in the formation, growth and guidance of pollen tube

Present investigation also revealed the presence of calcium in the mid of the colpus area. This area corresponds to the intine region from where the pollen tube emerges upon pollen hydration. Fluorescence microscopic observations in *Gasteria verrucosa* have also earlier revealed accumulation of calcium near the colpus area of the pollen (36). Calcium is important for the maintenance of rigidity of pectin in the intine (37). An accumulation of BPC1 and APC (calcium-binding proteins in *B. napus* and *Arabidopsis* respectively) has been reported on the surface of mature pollen grains of *Brassica napus* and *Arabidopsis* sp. (38). Calcium ions are known to be taken up from the stigma surface during pollen germination. Calcium channels have also been localized in the germinal aperture of pollen grains, which play a role in influx of calcium, upon hydration (39). Calcium enters the pollen tube at the tip and is then taken in by a sink consisting largely of endoplasmic reticulum (40). A sharp tip to base gradient of cytoplasmic calcium (3 μM to 0.2 μM) is required for the growth of pollen tube. A gradient of calcium has been recognized to play a significant role/s in the cytoskeletal reorganization and dynamics, exocytosis and endocytosis (41). Boron is known to cross link with pectic polysaccharides through a borate-diol ester, forming a pectin network (Kobayashi et al. 1999). It also forms a cross linked network with hydroxyproline rich glycoproteins (HRGPs) (42).

Concurrent with the present investigations, pollen grains are known to accumulate high levels of boron in the cytoplasm in forms that can be readily remobilized with carbohydrates and phenolics, which is then used for promoting the growth of pollen tubes. Boron deficiency causes a lowering of ATPase activity in the plasmalemma of chickpea root cells and in the pollen grains of lily. It has been suggested that calcium stabilizes the boron-pectin complex through ionic and coordinate bonds (43). Reproductive events like anther and pollen development, biochemical signaling in stigma for pollen recognition, pollen germination, and pollen tube growth, are known to be dependent on the availability of boron. Consistent to present studies, earlier studies have indicated that bee pollen have been known to have Na, K, Ca, Mg, P, Fe, Mn, Zn and Cu invariable concentration which are essential for the bee and human nutrition (Taha 2015)

Biomolecules present in pollen

The intine of the pollen is formed of pectins and cellulose, and is capable of stretching and contraction. The intine serves as a storage site for various hydrolytic enzymes. This includes the various peroxidases and esterases. The proteins of intine are gametophytic in origin and are stored in thick region near aperture (44). Carbohydrates serve as major energy source for germination, providing resistance to pollen dehydration, temperature stress and pollen viability. Various arabinogalactan proteins (AGPs) have been associated with the regulation reproductive success in plants. Entomophilous pollen have a lipid rich pollen coat. Pollen coat provides species specificity and mainly consists of neutral lipids, esterified by saturated fatty acid derivatives and volatile compounds, pigments and various proteins, filled in the cavities of exine. The components of pollen coat are required for pollen hydration and cellular communication between stigma and pollen. On successful pollination, the pollen coat material flows out from between the columellae of the exine to form an “attachment foot” on the stigmatic papillae. This is site of cellular communication between the pollen and stigma. Pollen proteins have a critical structural and functional role. Pollen coat proteins (PCP) have a role in pollen hydration and self-incompatibly signaling events. Pollen protein and lipids stored as lipid droplets polarize near the germinative aperture and then move to the pollen tube. These lipid droplets play an important role in signaling pathways, cytoskeleton dynamics and vesicle transport leading to successful pollen tube growth (2). Pollen proteins also serve as important dietary supplements for the pollinators. Sunflower pollen reduce protozoan pathogen (*Crithida bombi*) infection in bumble bee and microsporidian pathogen *Nosema ceranae* in *Apis mellifera* (45). Pollen grains contain a range of saturated and unsaturated fatty acids. In *Brasssica napus*, the pollen coat consists of 36% unsaturated fatty acid fraction and 57% saturated fatty acid fraction. In sunflower, unsaturated fatty acids consist of 55% of total fatty acid fraction and 30% saturated fatty acids. Linolenic acid is the major unsaturated fatty acid in the pollen. Among the saturated fatty acids, palmitic acid is a major constituent (46). In another report on sunflower, cis-11-eicosenoic acid (20:1) has also been reported as the major fatty acid in the pollen grains (Schulz et al. 2000). Lignoceric acid (24:0) has been reported to be present in the pollen coat, indicating its involvement during the hydration of the pollen grains on the stigma surface. Unsaturated fatty acids, like linolenic acid, produce signaling molecules, like jasmonates (McConn and Browse 1996).

In Arabidopsis, mutants defective in the synthesis of long chain lipids fatty acids fail to hydrate on the stigma surface, thereby showing the involvement of long chain fatty acids in cell-cell recognition required for hydration. As soon as stigma and pollen come in contact with each other, there is a mixing of pollen coat and stigmatic cuticle lipids (47). A polarization of lipids present in the pollen also takes place for the directional growth of the pollen tube into the stigma. Water moves by the capillary action, following the enzymatic activity between pollen and stigma surface, and changes in lipid composition of stigma cuticle are also brought about.

The pollen pigment and flavonoids present in the pollen coat provide protection to the pollen against photooxidative damage by UV and also provide it defense against various microbes. The antibacterial properties of pollen have been investigated by several researchers, and concurrent to present investigation, best inhibitory properties of sunflower pollen extract have been found against *Eschericihia coli, Staphylococcus aureus, Enterobacteriaceae and Pseudomonas aeruginosa*. Enrofloxacin, also known as 1-cyclopropyl-6-fluoro-7-(4-ethyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid, exhibits broad-spectrum antimicrobial properties, effectively targeting a wide range of both gram-positive and gram-negative bacteria. Functioning as a fluoroquinolone, it acts as an inhibitor of DNA Topoisomerase II and DNA Topoisomerase IV, disrupting crucial processes such as DNA replication (48). This drug is commonly utilized for treating various skin ailments and infections within the urinary tracts of diverse animal species. Recent findings suggest that secondary metabolites present in sunflower pollen extract demonstrate superior antimicrobial efficacy when compared to the standard antibiotic, enrofloxacin. The results imply that these secondary metabolites may reach microorganisms, influencing the properties of their membrane or disrupting the bacterial replication process. This proposition indicates a potential mechanism by which these secondary metabolites contribute to the elimination of both gram-positive and gram-negative bacteria. (49).

To sum up, cytochemical and histochemical qualitative test show the pollen coat and exine of pollen of *Heliathus annuus* is rich in lipids, proteins and polyphenols. These biomolecules form the attachment foot on the stigmatic papillae on successful pollination and initiate signaling events leading to the formation of polarized pollen tube. Carbohydrates, enzymes and biogenic elements present in the intine of pollen grain, namely potassium and calcium, have a role in harmomegathy and cause physiochemical changes leading to directional growth of pollen tube. Polyphenols present on the pollen grains provide them antioxidant and antimicrobial properties. Further studies in this field would be important for pollination biology, crop improvement and conservation programs that aim at coevolution and maintenance of ecological services

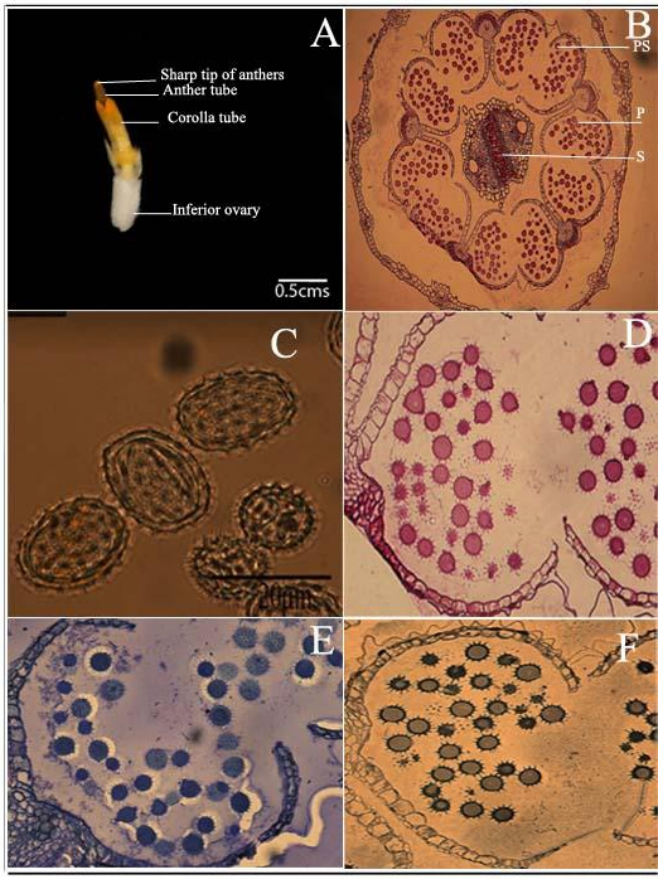

Figure 1: *A: Disc floret of sunflower, staminate stage showing the presence of syngensious anthers B: Transverse section of the floret showing the presence of stigma (S), pollen sacs (PS) and pollen (P) Magnification: 100X. C: Pollen grains in Sunflower. Magnification: 630X. D-F: Transverse section of pollen sacs showing the presence of carbohydrates, in the intine (IN) and exine (EX) of pollen respectively. Magnification:400X.*

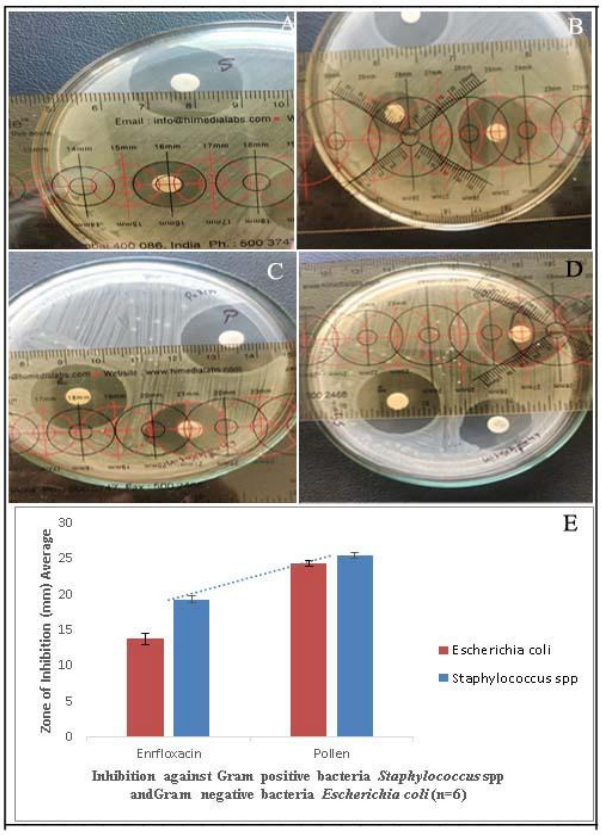

Figure 2: *A: Zone of inhibition showing antibacterial activity of enrofloxacin (control) against E. coli. B: Zone of inhibition showing antibacterial activity of pollen extract against E. coli. C: Zone of inhibition showing antibacterial activity of enrofloxacin (control) against Staphylococcus spp. D: Zone of inhibition showing antibacterial activity of pollen extract against Staphylococcus spp. E: Statistical analysis revealing zone of inhibition of pollen extract against. E. coli and Staphylococcus spp. as compared to standard drug, enrofloxacin.*

Conflict of Interest

The authors declare that there are no conflicts of interest.

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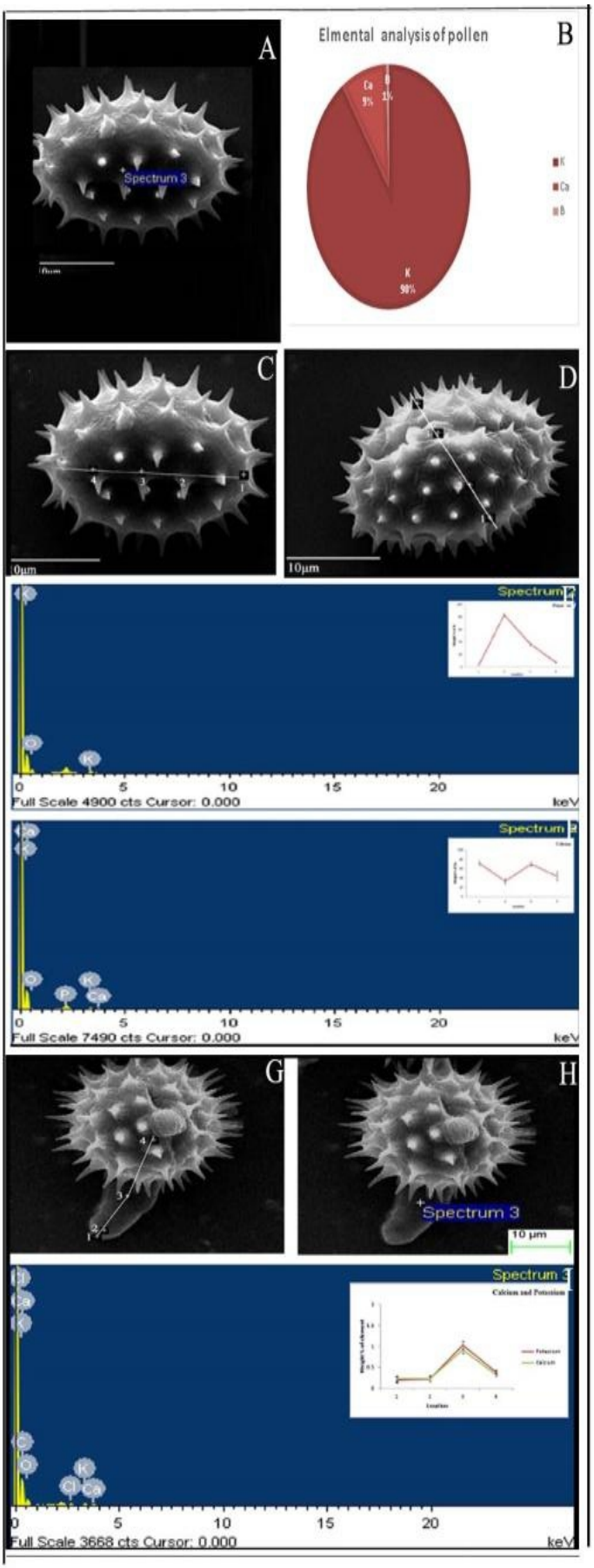

Figure 4: *A: Pollen grain, on dispersal as observed through EDAX. B: Quantitative estimation of potassium, calcium and boron in pollen as estimated by flame photometer. C,E: Localization of potassium by energy dispersive X-ray analysis (EDAX) Inset(E): Quantification of calcium. D,F: Localization of calcium by energy dispersive X-ray analysis (EDAX) Inset(F): Quantification of calcium. G: Germinating pollen grain as observed by EDAX. H, I: Localization of calcium and potassium on germinating pollen by energy dispersive X-ray analysis (EDAX). Inset: Quantification of calcium and potassium.*

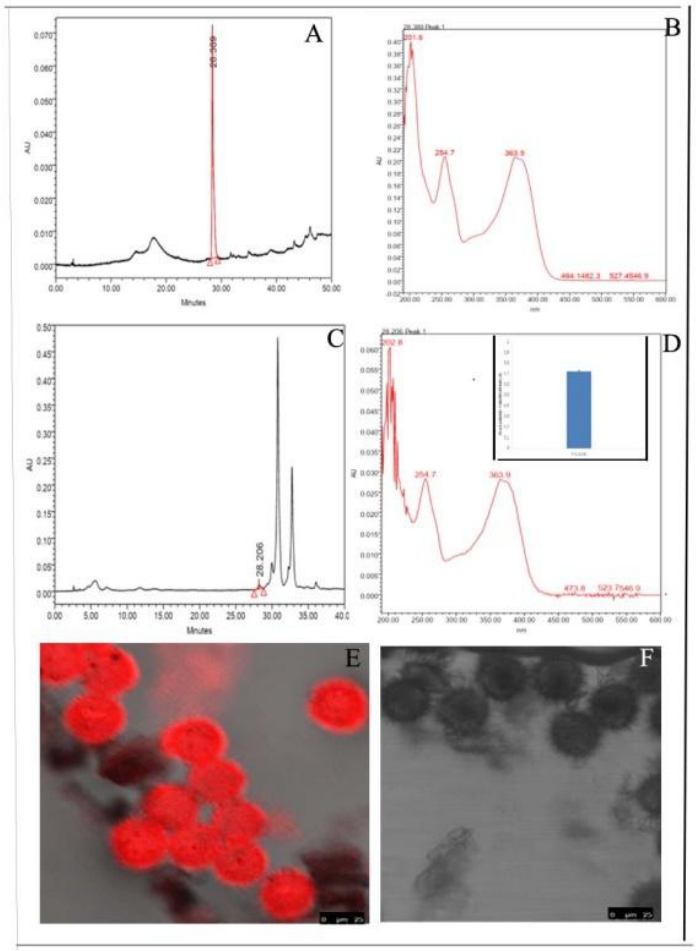

Figure 3: *A: HPLC chromatogram obtained by using standard quercetin. B: Excitation/Emission wavelength obtained for standard quercetin. C: HPLC chromatogram obtained for pollen extract. D: Excitation/Emission obtained for pollen extract, Inset-showing the quantitative estimation of quercetin present in pollen extract. E-F: Localization of polyphenols on pollen of sunflower using the stain (diphenylboricacid 2-aminoethylester), following UV excitation at 405 nm and emission of 580 nm as compared to the control.*

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