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Anti-bacterial efficacy of bee pollen samples collected from Chandigarh (U.T.) during spring season

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ABSTRACT

The study was conducted in Chandigarh during spring season to find the potentiality of flora with high anti- bacterial efficacy. Mainly four bacterial strains [*Pseudomonas aeruginosa* (MTCC 2453), *Klebsiella pneumoniae* (MTCC 109), *Streptococcus pneumoniae* (MTCC 655) and *Salmonella typhimurium* (MTCC 3231)] were selected for the study. First pollen aqueous extract (PAE) of different concentrations (20%, 40%, 60% and 80%) were prepared, then disc diffusion method was used to check the anti- bacterial efficacy. *Pseudomonas aeruginosa* was highly inhibited by bee pollen of sub- divisions 1, 2, 3 and 4.Sub- division 3 and 4 also has higher action against *Salmonella typhimurium.* Sub- division 5 has higher efficacy against *Klebsiella pneumoniae*. The study throws light on their therapeutic use of bee pollen.

Key words: *Bee pollen, Anti- bacterial efficacy, Pollen aqueous extracts (PAE), Spring season, Therapeutic use*

Introduction

Pollen is male gametophytic part of flower, when honey bees visit flower to collect nectar, the pollen dust gets attached to their body and transferred to another flower by pollination. Honey bees collect pollen dust particles into their pollen basket present on hind legs. The collected pollen is known as 'bee pollen'. Bee pollen is the agglutination of plant pollen with nectar and salivary secretion of worker honey bee (Campos *et al.*, 2008). It is the sole source of protein in the hive for the rearing of brood and the development of the colony. It also supports worker bees to secrete royal jelly for rearing brood. Pollen consists of various bioactive compounds like carbohydrates, proteins, amino acids, vitamins, carotenoids and traces of micronutrients (Bonvehi and Jorda, 1997). Their variable number largely depends on the botanical and geographical origin of bee pollen. Due to its high nutritional properties, it is used in the food industry and also considered special food (Bogdanov, 2004). Bee pollen has various therapeutic properties such as anti-bacterial, anti-fungal, anti-carcinogenic and immunomodulatory. Pollen can be used to determine insect migration, insect food sources, honey types and forensics because of its distinctive nature and presence of sporopollenin which is a durable chemical substance (Jones and Jones, 2001).

Pollens of some plant species are greatly preferred by honey bees over other types of pollen species which mainly depends on the shape of the outer surface of pollen, the chemical composition of pollen and the availability of forage sources near hives. Presence of different phyto- chemicals mainly phenolic compounds in pollen greatly affects their anti-bacterial properties (Almeida- muradian *et al.*, 2005).

In this study, bee pollen of Chandigarh area collected during spring season was analysed to check their anti-bacterial efficacy against various bacterial strains by using disc diffusion method. Study was done to highlight the area of Chandigarh with very

efficient flora whose pollen has great action against particular bacteria.

Methodology

The present study focuses on the anti- bacterial efficacy of bee pollen collected from Chandigarh during spring season.

Study Area

The present study was carried out in 5 sub- divisions of Chandigarh area (Table 1) which were marked by Horticulture department, Municipal Corporation, U. T., Chandigarh. Colonies were installed and maintained in each sub- division.

Study Period

Study was done during spring season (mid February to early April). Observations were done thrice a day i.e. morning: 700-800 hrs, afternoon: 1300-1400 hrs, evening: 1700-1800 hrs.

Sample Collection

Pollen traps were installed at the entrance of Langstroth bee hive to collect bee pollen samples. Collected samples were preserved at -20°C for future use in air tight glass containers.

Anti- Bactertial Efficacy Evaluation

Mainly four pathologically harmful bacterial strains [*Pseudomonas aeruginosa* (MTCC 2453), *Klebsiella pneumoniae* (MTCC 109), *Streptococcus pneumoniae* (MTCC 655) and *Salmonella typhimurium* (MTCC 3231)] were selected to test the anti-bacterial properties of the bee pollen. All these bacterial strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh. Agar disk diffusion method was used to check the anti- bacterial efficacy of bee pollen samples by measuring zones of inhibition against different bacteria. The anti-bacterial efficacy was expressed in terms of the diameter of zones of inhibition in centimetre (cm) (Ponce *et al.,* 2003 and Moreira *et al.,* 2005). All the experiments were repeated in triplicates for all the microbial strains.

Anti- microbial discs of Tetracyclin and Streptomycin were used as positive control.

Preparation of Bee Pollen Extracts

Pollen water extracts of different concentrations (20%, 40%, 60% and 80%) were prepared. All the samples were also filtered through micro syringe filters in laminar air flow to make the samples contamination free. After filtration samples were stored at -20^{0}C for further studies.

Anti- microbial discs of Tetracyclin and Streptomycin were used as positive control.

Statistical Analysis

All experiments were done in triplicates. The average of all the results obtained from anti-bacterial tests was taken as the final value. All the values were expressed as Mean ± S.D.

Results

Anti-bacterial efficacy is the tendency of any substance to kill the bacteria. Pollen also has natural tendency to kill bacteria. In this study, anti-bacterial efficacy of pollen samples collected from different sub- divisons in spring season were analysed to identify the area which has flora with greatest anti-bacterial efficacy against different studied pathogenic bacteria.

Different concentrations (20%, 40%, 60% and

Table 1. 5 sub- divisions of Chandigarh

S. No.	Sub- Divisions	Area Details
1	Sub-division 1	SECTOR- 25, 36-42, Attawa, Buterla, Dhanas, Dadumajra, Maloya Colony, Janta Colony.
2	Sub-division 2	SECTOR- 27-32, Industrial area phases 1 and 2, Sanjay Colony (SLUM), Karson Colony, Bair-majra.
3	Sub-division 3	SECTOR- 1- 19, 21-24 and KhudaLahora.
4	Sub-division 4	SECTOR- 26, Bapudhamtragt Camp I, II, Phase I, II,EWS Housing Board, Bapudhamtragt Camp III Phase III and Madarsi Colony (HUTS), Mouli Complex, Bhagat Singh Colony, Vikas Nagar, Mohalla Govindpura, Dhillo Complex, Motor Market pocket no. 8, Darshani Bagh, Subhash Nagar, Adarsh Nagar, Pipli Wala Town, Gawala Colony, Indira Colony (Reh.), Old Indira Colony Race Course and I.T. park.
5	Sub-division 5	SECTOR- 20, 33-35, 43-45, 49-52 (Nizam Pur Burail), Brick- Killin, Nizampur Kumbra, Sub jail, Nimpur Kumbra and Sec- 55, 56, 60, 61, 63, and Burail

80%) were prepared just to check that at which level of concentration samples show highest anti-bacterial property. This property of the samples can be used against different bacteria to treat various dieases. Tetracyclin and Streptomycin discs were used as positive control. These discs have great anti-bacterial action against all studied bacteria. Tetracyclin showed highest anti-bacterial efficacy than Streptomycin against all bacteria except *Salmonella typhimurium*. In case of *Salmonella typhimurium* Streptomycin showed good ant-bacterial efficacy. Each sub- division has different flora and these floras have their unique pollen with distinguished biological properties. Results of different samples against different different bacteria are shown below:

Test against *Pseudomonas aeruginosa* (Table 2)

PAE of spring season

During spring season, samples of sub- divisions 1 and 3 presented the minimum zone of inhibition and maximum by samples of sub- division 2 at 20 % concentration. At similar concentration, samples of subdivisions 4 and 5 showed similar results. 40% PAE of sub- divisions 2 and 4 showed maximum value of zone of inhibition and minimum by sub- division 3 samples. At similar concentration, samples of sub- divisions 1 and 3 have similar results. 60% PAE of sub- division 2 has maximum zone of inhibition and of sub- division 1 has minimum value. Results of sub-divisions 3 and 4 have similar values similar concentraton of 60%. At 80% concentration, PAE of sub- division 1 has maximum value. PAE of sub- divisions 3 and 5 have similar effect against *Pseudomonas aeruginosa* during spring season at 80% concentration. From all the results, it was found that sub- division 2 has good anti-bacterial efficacy as compared to other sub- divisions.

Test against *Klebsiella pneumoniae* (Table 2)

PAE of spring season

During spring season samples of Sub- division 1 have highest values of zone of inhibitions at 20%, 40%, 60% and 80% PAE. Sub- division 2 samples have less susceptibility against this bacteria at all studied concentration of PAE. At 20% sub- divisions 4 and 5 have similar results. 80% PAE of sub- division 3 and 4 also shared common values of zone of inhibitions.

Test against *Salmonella typhimurium* (Table 2)

PAE of spring season

20 % PAE formed greatest zone of inhibitions with samples obtained from sub- divisions 2 and 3. Least zone of inhibition was formed by 20% PAE of sub- division 5. 40% PAE of sub- division 4 has least anti-bacterial action against *Salmonella typhimurium* but of sub- division 2 and 5 has greater anti-bacterial efficacy against this bacteria. 60% PAE and 80% PAE of sub- division 5 also have good anti-bacterial properties against *Salmonella typhimurium.* Sub- divisions 1 samples were found to have less anti-bacterial action against this bacteria at concentrations 60% and 80%.

Table 2. Anti- bacterial efficacy of pollen acqous extracts of different concentrations against bacterial strains.

	Pseudomonas aeruginosa				*Klebsiella pneumoniae*				*Salmonella typhimurium*				*Streptococcus pneumoniae*			
PAE	20%	40%	60%	80%	20%	40%	60%	80%	20%	40%	60%	80%	20%	40%	60%	80%
Sub- div. 1	0.9±0.28	1.1±0.14	1.15±0.49	1.85±0.21	0.8±0.28	1.1±0.28	1.1±0.14	1.25±0.07	0.6± 0.84	1.15±0.21	1.4±0.07	1.55±0.07	0.6±00	0.85± 0.07	0.8± 0.28	1.05±0.07
Sub- div. 2	1.05±0.07	1.25±0.07	1.5±0.14	1.75±0.07	0.85±0.21	1.15±0.21	1.15±0.07	1.45±0.07	1.15 ± 0.35	1.25±0.07	1.35±0.21	1.45±0.07	0.85± 0.07	1.1± 0.14	1.1± 0.14	1.35±0.21
Sub- div. 3	0.9±0.14	1.05±0.07	1.3±0.14	1.4±0.14	0.85±0.21	0.95±0.07	1.1±0.14	1.25±0.07	0.95 ± 0.07	1.45±0.21	1.25±0.35	1.4±0.28	0.85± 0.21	1.05± 0.35	1.1± 0.14	1.25±0.35
Sub- div. 4	0.95±0.07	1.25±0.07	1.3 ±0.14	1.5±0.14	1.05±0.21	1.05±0.07	1.1±0.14	1.35±0.07	0.95 ± 0.21	1.25±0.07	1.4±0.14	1.85±0.21	0.65± 0.07	0.75± 0.07	0.95± 0.21	1.25±0.03
Sub- div. 5	0.95±0.21	1.1±0.28	1.2±00	1.4± 0.14	0.9±0.28	1.2±0.42	1.3±0.42	1.45±0.49	0.8± 0.14	1.05±0.21	1.15±0.21	1.3±0.14	0.35± 0.49	1.1± 0.28	1.2± 0.28	1.35±0.21

Test against *Streptococcus pneumoniae* (Table 2)

PAE of spring season

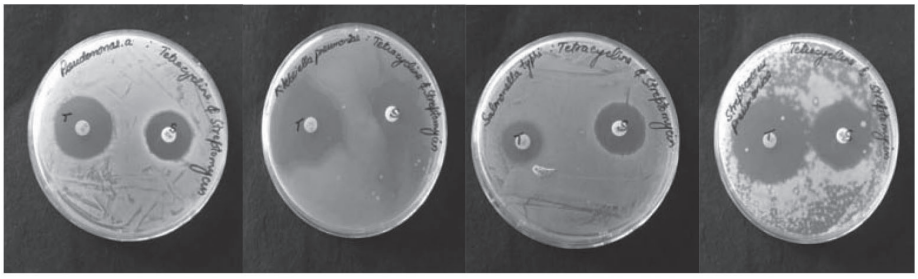
During spring samples of sub- division 5 has highest while sub- division 3 and 1 have overall lowest anti-bacterial action against *Streptococcus pneumoniae bacteria* at different concentrations. Sub- division 1 formed minimum zone of inhibition at 20%, 60% and 80% concentration as compared to samples of other sub- divisions. 20% PAE od sub- divisions 2 and 3 shared values. Sub- division 5 formed small zone of inhibition at 40%, 60% and 80% concentrations.

Discussion

Various anti- microbial tests were also done against honey bee products to know their anti- microbial susceptibility. In anti-bacterial study of Ozkalp and Ozcan (2010), anti-microbial activity of pollen and propolis against various bacteria (*Streptococcus salivarius, Listeria monocytogenes, Streptococcus aureus, Salmonella enteritidis, Staphylococcus pneumoniae, Escherichia coli, Klebseilla pneumoniae, Pseudomonas aeruginosa* and *Bacillus anthracis*) and found that propolis extract has higher inhibitory effect than the pollen. At concentrations (conc.) 400 ppm and 600 ppm propolis extract has inhibitory results against *Listeria monocytogenes* as compared to amikacine, gentamicine and ceftriaxone antibiotis. At higher conc. of 600 ppm propolis extract showed similar inhibition against *Staphylococcus aureus*. Our results of anti-bacterial efficacy test showed the increased inhibition with increase of dosage against five tested pathogenic bacteria. Similar results were also found by Ozkalp and Ozcan (2010), they also represented the increase in inhibition of both pollen and propolis extract by increasing their dosage against all tested bacteria. Previously it was also found that the polar phenolic compounds are mainly responsible for anti-bacterial activity of any compound (Bankova *et al.,* 1995). In various tests, anti-bacterial activity of pollen was compared with propolis in which propolis showed the highest inhibition (Ozkalp and Ozcan, 2010).

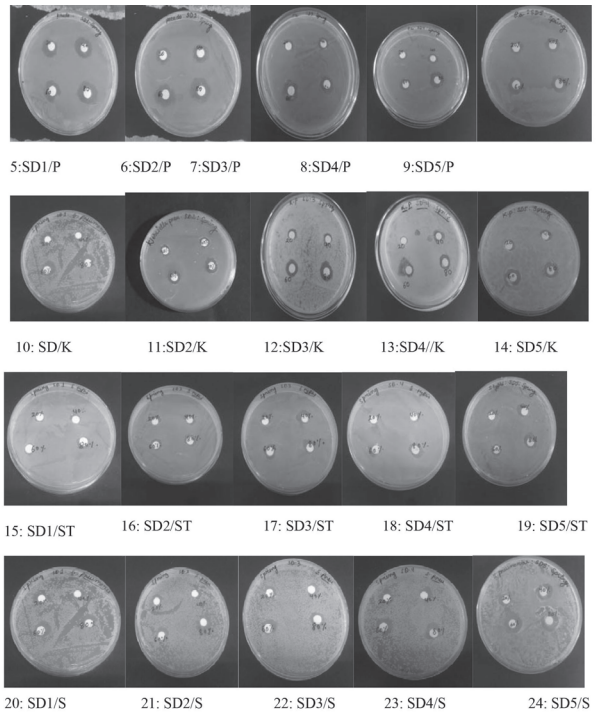
In anti-bacterial study of Eswaran and Bhargava (2014), pollen ethanolic extract (PEE) and pollen aqueous extract (PAE) were prepared of 50 % and 90% conc. They used disc diffusion method (Carpes *et al.,* 2007) and observed greater inhibition of 50% PEE against *Salmonella typhi* and least on *Klebsiella pneumoniae.* It was also effective against *Pseudomonas aeruginosa*. 90% PEE had greater inhibition against *Pseudomonas aeruginosa* which was followed by *Salmonella typhi* and *Proteus mirabilis* and also form less zone of inhibition against *Bacillus subtilis*.

PAE showed high inhibition against *Pseudomonas aeruginosa*. Both extracts, PAE and PEE showed the good inhibition against *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis* but least inhibition against *Bacillus subtilis*. One thing among all the previous and present studies of anti-bacterial efficacy of pollen was found that inhibition of pollen extract was increased with increasing conc. So, here 90% PEE exhibited higher inhibition against bacteria as compared to extracts of lower concentration. PEE also showed the higher levels of phenolic compounds (>10 mg/g) at 60, 70 and 80 % of concentration. These concentrations did not present any significant difference between the extraction conditions (solvent conc.) but conc. 40, 50 and 90% showed statistical difference. The variations in the activity of pollen extract against different bacteria are due to different phenolic compounds in pollen which are



1: Tn & Sn/P 2:Tn & Sn/K 3: Tn & Sn/ST 4:Tn & Sn/S

Plates 1-4. Control (Action of Tetracycline and Streptomycin (T & S) drug against different bacteria (*Pseudomonas aeruginosa* (P), *Klebsiella pneumoniae* (K), *Streptococcus pneumoniae* (S) and *Salmonella typhimurium* (ST))



Plates 5-24: Anti- bacterial efficacy of bee pollen samples collected from sub- divisions 1-5 (SD1-5) during spring (SP) against different bacteria (P/K/S/ST)

mainly dependant on the source of pollen (Almeida-Muradian *et al.,* 2005). Each plant has its specific type of phenolic compounds in specific amount.

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