<u>Original Research Article</u>

Morphology, Cytology and Ethnobotany of three *Pimpinella* L. species (Apiaceae) from the North-Western Himalayas, India

ABSTRACT:

During the present study, population-based meiotic studies were carried out on three species and 14 populations of the genus *Pimpinella* from selected localities of district Kangra and Sirmaur of Himachal Pradesh in the North-Western Himalayas (India).The present chromosome adds up of 2n=18 in *P. acuminata* making acount of new diploid cytotype on a world-wide basis.Impaired male meiosis was eminent in all populations of *Pimpinella achilleifolia* and *P. diversifolia* and the populations with 2n=18 in *P. acuminata*. These uneven species were striking with meiotic abnormalities in the form of cytomixis, chromosomal stickiness, unoriented bivalents, formation of laggards and bridges consequential in abnormal microsporogenesis, and production of heterogeneous-sized fertile pollen grains along with reduced pollen fertility.

KEY WORDS: Chromosome number, Cytomixis, Meiotic course, Morphology, Ethnobotany, Pollen fertility, *Pimpinella*.

INTRODUCTION

The *Pimpinella* genus is a group of plants within the Apiaceae family, which is commonly known as the carrot or parsley family. This genus encompasses a diverse range of aromatic herbs, many of which have been historically used for culinary, medicinal, and ornamental purposes. The genus is widespread and includes both annual and perennial 170-180 species [1]. In India, it is represented by 30 species [2].*Pimpinella* genus includes compound leaves, often finely divided, and umbrella-shaped flower clusters known as umbels. The plants within this genus are typically characterized by their aromatic properties, owing to the presence of essential oils in their tissues [3].

Noticeable variations in the *Pimpinella* genus include fruit shape (ovoid-oblong to elliptic), fruit surface (glabrous to tuberous hair), fruit anatomy, leaf division, flowers and inflorescences, which result in the identification of several and often scarcely evident taxa [4]. Basal leaves and mature fruits are two important factors required for accurate identification.However, because of its enormous size, wide geographical range and elevated levels of intraspecific variation, the generic boundary of *Pimpinella* and its intrageneric relationships remain unclear [5]. To accomplish a steady taxonomy for this genus, it is not only essential to improve the account of morphological characters as well as their patterns of dissimilarities and distribution across species but also to test those characters against solid molecular phylogenetic study. The genus has been rarely studied from a morphological or phytochemical perspective [6]. The most inclusive phytochemical analysis of the genus was published by [7], who also described the phylogenetic associations among species. A recent molecular phylogenetic study of the genus showed it to be a non-monophyletic taxon [8,9] .

The ethnobotany of the *Pimpinella* genus is rich and diverse, with various species being utilized for different purposes in different cultures. Ethnobotany explores the relationship between plants and people, encompassing traditional knowledge, cultural practices, and the uses of plants for various purposes, including food, medicine, rituals, and crafts. *Pimpinella anisum* seeds are used as a spice in cooking, baking, and the production of beverages. *Pimpinella major* has been used in folk medicine for its diuretic and anti-inflammatory properties. *Pimpinella brachycarpa* has been used in traditional Chinese medicine for its potential anti-inflammatory and analgesic effects [10,11].

Cytological studies are valuable for assessing the genetic diversity within and among *Pimpinella* populations. This information is crucial for conservation efforts and understanding the potential for adaptation to changing environmental conditions. Previously, no attempt has been on cytology of *Pimpinella* genus so, main intention this paper provides a detailed description of chromosome number and comparative effects of chromatin transfer on the meiotic course, pollen fertility and size.

MATERIALS AND METHODS

In general, 10 randomly selected plants of each species/population were studied. Different qualitative morphometric characters were studied for each cytotype to have proper insight on morphological variation. For stomatal studies, mature leaves were treated with 10% aqueous solution of potassium hydroxide (KOH) at room temperature for 10–15 min and then epidermal peels so obtained were stained with 10% saffarnin in 90% ethanol. For meiotic studies, plant materials were collected from selected localities of different areas of the Western Himalayas. Usual acetocarmine smears of appropriate sized flower buds were made after fixing them in the Carnoy's fixative(6:3:1 ethanol/chloroform/acetic acid v/v/v) for 24 h and preserved in 70% ethanol at 4°C untilused. Pollen fertility was estimated by mounting mature pollen grains in glycero–acetocarmine(1:1) mixture. Well-filled pollen grains with stained nuclei were taken as apparently fertile, whileshrivelled and unstained pollen grains were counted as sterile. Pollen grain size was measured usingan ocular micrometre. Photomicrographs of pollen mother cells and pollen grains were madefrom freshly prepared slides using a Nikon 80i eclipse Digital Imaging System. Voucher specimensare available in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

RESULTS AND DISSCUSSION

Cytology

Three species, namely, *P. achilleifolia* DC., *P. acuminata* Clarke and *P. diversifolia* DC. have been cytologically explored at present.

P. achilleifolia **DC. (=** ***Ptychotis achilleifolia*** **DC.**) is a 30-100cm long perennial herb with leaves triangular-ovate, pinnate, held in one plane; petiole up to 20cm long; umbel terminal, 10-15 flowered. It is found as common species in moist places at an altitude of 2,300m-2,600m. Flowering and fruiting during the months of July-September. During meiosis, the PMCs uniformly exhibit the presence of 9 bivalents at Diakinesis (Fig. 1) and 9:9 chromosomes distribution at A-I (Table 1; Fig. 1-2). The present chromosome count of 2n=18 conformsto the previous single report from India [12].

P. acuminata **Clarke** is glabrous, except the leaves; stem 1-2m; leaves pubescent on both surfaces, 2-pinnate; leaflets 3-5 on each pinnule, irregularly lobed, toothed, long pointed; flowers yellow-green; stalks of fruits much elongated, drooping; fruits smooth. It is found as common species in moist places along roadsides between the altitudes of 1,400-2,400m. Flowering and fruiting are seen during the months of July-September.

At present six populations have been meiotically studied, four showing chromosome number 2n=18 and abnormal course whereas, one with 2n=20 and normal course. The chromosome count of 2n=18 is

confirmed from 9 bivalents at M-I (Fig. 9) making addition of new diploid cytotype on world-wide basis for the species. The report of 2n=20 counted from 10:10 distribution of chromosomes at A-I (Table 1; Fig. 10) is in conformity with the previous reports from India [13]and from outside India fromNepal [14]. The species is also known to exist as 2n=40 [13].

P. diversifolia **DC.** is an erect, pubescent, annual herb with stems corymbosely branched upwards; leaves nearly 2-8cm long (decreasing upwards); flowers white in compound umbel; fruits nearly 2mm long, narrowed towards the apex, laterally compressed; ridges distinct. It grows as a common plant on moist places, in wastelands between the altitudes of 1,000-2,700m. Flowering and fruiting are seen during the months of July-October.

The meiotic chromosome number of 2n=18 in the presently studied populations has been confirmed from the presence of 9 bivalents at Diakinesis/ M-I (Table 1; Figs. 18-19). This count is following the previous reports from India [15] and from outside India. The species is also known to have a cytotype of 2n=28 from the Hengduan Mountains of China.

All the above discussed ten populations of *P. achilleifolia* and *P. diversifolia* and two of *P. acuminata* show highly abnormal meiotic course. In such populations, abnormalities are in the form of cytomixis, unoriented bivalents, chromatin stickiness, chromatin bridges and laggards at different stages of meiosis (Table 2). The cytomixis involving transfer of chromatin between PMCs, is seen right from the Prophase-I to T-II stage (Figs. 3, 11, 20, 21, 22; Table 2). Other associated abnormalities includeunoriented bivalents, chromatin stickiness, chromatin bridges and laggards at different stages of meiosis (Figs.4, 5, 6, 12, 13, 14, 15;Table 2). Microsporogenesis leading to the formation of triads, tetrads or polyads with or without micronuclei is also seen in these populations (Figs. 7, 8, 16, 25; Table 3). Heterogeneous sized fertile pollen grains and reduced pollen fertility has also been observed in these taxa (Figs. 17, 26).

Table1: Data pertaining to Accession no., Habit, Location, Altitude, Chromosome no., Ploidy level, meiotic course, Pollen size and Previous chromosomal reports of the presently studied species of the genus *Pimpinella* L. (Apiaceae) from the north-western Himalayas, India.

Sr. No	Taxon (Accession number-PUN+)		Locality with Altitude (m)	Chrom-osome number (2n)	Ploidy Level/ Meiotic course*	Pollen Fertility (%)	Pollen size (µm)	Previous reports***
1.	***Pimpinella achilleifolia* DC.**							
	P-1	52701	Chotta-bhangal (Distt. Kangra)/ 2,300	18	2x/N	90.00	23.54×18.99	**2n=18 Bhattacharya 1967**
	P-2	52716	Lohardhari(Distt . Kangra) 2,600	18	2x/A	60.73	23.00×19.09 21.33×18.00	
	P-3	56761	Chapdhar (Distt. Sirmaur) 2,600	18	2x/A	80.24	22.37×17.76- 18.21×13.37	
2.	***P. acuminata* Clarke**							
	P-1	55152	Lohardhari(Distt . Kangra)2,600	20	2x/N	98.89	23.55×20.89	**2*n*=20,40 Mehra & Dhawan 1971;**Carbonnier & Farille 1980; Cauwet-Marc 1982
	P-2	55248	Rajgarh, Distt. Sirmaur)1,650	18**	2x/A	70.50	24.20×13.18- 22.53×12.27	
	P-3	55888	Kotli, Distt. Sirmaur)1,600	20	2x/N	98.16	23.28×14.18	
	P-4	55889	Dedag, Distt. Sirmaur)1,800	18**	2x/A	80.12	24.38×13.09- 20.33×12.28	
	P-5	55890	Baru-Sahib, Distt. Sirmaur)1,800	18**	2x/A	77.78	23.09×12.78- 21.11×11.88	
	P-6	55891	Nauradhar, Distt. Sirmaur)1,800	18**	2x/A	76.45	24.12× 13.26- 21.45×13.12	
3.	***P. diversifolia* DC.**							
	P-1	55146	Chotta-bhangal(Distt. Kangra) 2,300	18	2x/A	62.78	24.87×18.77 - 22.00×19.44	**2*n*=18 Sharma & Bhattacharyya 1959**; Liu *et al.* 1961; **Ahmad & Koul 1980**; Pimenov 1999; **Pimenov *et al.* 2006** **2*n*=28** Pu 2006
	P-2	52662	Rajgarh, Distt. Sirmaur) 1,650	18	2x/A	60.48	21.18×12.28- 19.29 ×11.76	
	P-3	55879	Kotli, Distt. Sirmaur)1600	18	2x/A	61.78	22.46×14.27 18.65 ×13.45	
	P-4	55880	Baru-Sahib, Distt. Sirmaur)1,800	18	2x/A	73.32	22.76×14.18- 18.00 ×12.17	
	P-5	55881	Nauradhar Distt. Sirmaur)1,800	18	2x/A	76.22	23.16×13.58- 19.03 ×12.59	

**New intraspecific cytotypes recorded on world-wide basis. N= Normal, A=Abnormal (Meiotic course).

Table 2: Data on meiotic abnormalities in different species of *Pimpinella*.

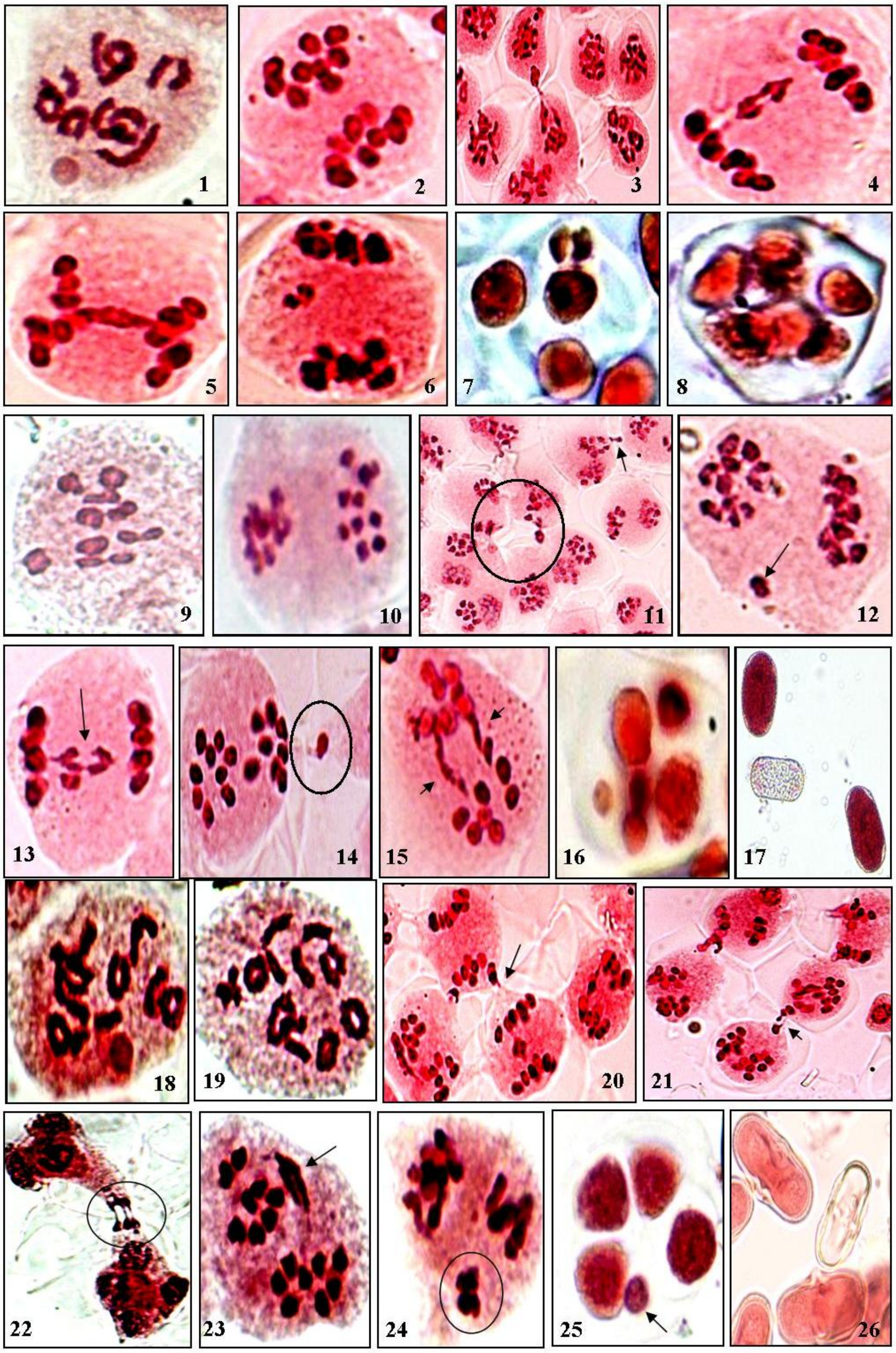
Accession number	**Cytomixis**		**Meiotic course showing PMCs with**		
	% of PMCs invol at Meiosis-I/Meic	Number of F involved	Chromosomal stickiness at M-I (%)	Bridges at Meiosis I/Meiosis-II (%)	Leggards at Meiosis-I/ Meiosis-II (%)
P. achilleifolia					
56761	5.20 (5/96)/ 2.59(2/77)	2–3	----	3.70(3/89)/----	7.14 (6/84)/ 3.48 (3/86)
52716	----/----	2–3	4.22(6/142)	3.64(5/137)/----	4.96(7/141)/---
P. acuminata					
55248	7 .37 (9/122)/ 2 .22 (2/90)	2–3	----	4.44(4/90)/ 1.21(1/82)	4.12 (4/97)/ 1.16 (1/86)
55889	6.60 (7/106)/----	2–4	----	----/---	6.08 (7/115)/ 1.19(1/84)
P. diversifolia					
52662	12.80 (16/125)/ 4.44(4/90)	2–4	6.41 (5/78)	2.67 (3/112)/---	10.18 (11/108)/ 2.75(4/145)
55146	----/----	----	2.96(4/135)	4.31(6/139)/----	4.92(7/142)/---
55879	5.95 (5/84)/ 4.28(3/7	2–5	7.40(6/81)	----/----	5.35 / (6/112)/ 2.50(2/80)
55880	9.57 (9/94)/ ---	2–3	----	3.27(4/122)/---	4.59 (4/87)/ 3.40 (3/88)

Figures in parenthesis denote observed number of abnormal PMCs in the numerator and number of PMCs observed in denominator.

Table 3: Data on abnormal microsporogenesis in different species of *Pimpinella.*

Accession number	Microsporogenesis (Values in %)				
	Triads		Tetrads		Polyads
	WMN	WM	WMN	WM	
P. achilleifolia					
56761	3.49(5/143)	4.89(7/143)	80.41(115/143)	8.39(12/143)	2.79 (4/143)
52716	9.09(9/99)	2.02(2/99)	75.75(75/99)	12.12(12/99)	--
P. acuminata					
55248	4.83(6/124)	1.61(2/124)	86.29 (107/124)	7.25(9/124)	---
55889	4.39(4/91)	----	86.81 (79/91)	6.59(6/91)	2.19 (2/91)
P. diversifolia					
52662	5.50(6/109)	3.66(4/109)	81.65 (89/109)	3.66(4/109)	5.50(6/109)
55146	3.03(3/99)	4.04(4/99)	84.84 (84/99)	8.08(8/99)	----
55879	3. 05(4/131)	----	87.78 (115/131)	5.34(7/131)	3.81 (5/131)
55880	4.04(4/99)	1.01(1/99)	79.79(79/99)	15.15(15/99)	---

Figures in parenthesis denote observed number of abnormal PMCs in the numerator and number of PMCs observed in denominator.
WMN without micronuclei, *WM* with micronuclei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Figs. 1-8: *Pimpinella achilleifolia* DC*.*

Figs. 1-8: Meiosis in PMCs (2n=18)

Fig. 1: Diakiesis showing $9_{II.}$

Fig. 2: A-I showing 9:9 distribution of chromosomes.

Fig. 3: Group of PMCs showing cytomixis.

Fig. 4: Late disjunction of bivalents at A-I

Fig. 5: Bridge at A-I.

Fig. 6: Laggard at late A-I.

Fig. 7: A tetrad with micronuclei.

Fig. 8: A polyad.

Figs. 9-17: *P. acuminata* Clarke

Figs. 9-17: Meiosis in PMCs (2n=18 & 20)

Fig. 9: M-I showing $9_{II.}$

Fig. 10: A-I showing 10:10 disttribution of chromosomes.

Fig. 11: Group of PMCs showing cytomixis.

Fig. 12: Laggard at A-I.

Fig. 13: Late seaparation of bivalent at A-I.

Fig. 14: Transfer of chromosome at A-I

Fig. 15: Bridge at A-I.

Fig. 16: A polyad.

Fig. 17: Fertile and sterile pollen grains.

Figs. 18-26: *P. diversifolia* DC.

Fig. 18-26: Meiosis in PMCs (2n=18)

Fig. 18: Diakinesis showing $9_{II.}$

Fig. 19: M-I showing $9_{II.}$

Figs. 20-22: Group of PMCs showing cytomixis at A-I and T-II.

Fig. 23: Laggards at A-I.

Fig. 24: Extra chromatin mass at late A-I.

Fig. 25: A tetrad with micronucleus.

Fig. 26: Fertile and sterile pollen grains.

Chromosome numbers and morphology

The genus is characterized by basic chromosome numbers as x = 8, 9, 10, 11 with 66 species cytologically known on the world level (2n=16, 18, 20, 22, 24, 28, 36, 40) and 6.06% showing polyploidy. Out of 30 taxonomically known species from India, all the 9 (30.00%) cytologically worked out species are diploids (2n=18, 20, 22). In all, 7 species are marked with euploid series based in x= 9 and / or 10 and 21 depict intraspecific aneuploidy. Some aneuploid species have been reported [16,17]. In various cases, intraspecies and even intrapopulation discrepancy has been originated in the ploidy level [17]. Many *Pimpinella* species are barelydispersed and habitat-specific [18]. Therefore, this indicates that the process of speciation in this genus is tightly associated with polyploidy and adaptation, and the evolutionary history of *Pimpinella* is not been well understood.

The chromosomal variation (aneupliod cytotypes) in the species is coupled with morphological variation as both the cytotypes are clearly distinct in the field. Some significant morphological variations noticed between the two cytotypes are listed in Table 4. Aneuploidcytotpes are usually taller than their diploid counterparts [19]. At present, it was observed that all the individuals of aneuploid cytotype (2n=20) are almost double in height compared to the diploid ones. On the leaf morphological characters, it was seen that number of leaves/plant, stomata size and stomatal frequency generally depicted higher values for the aneuploid individuals than the diploid ones. Therefore mild gigantism for some quantitative and qualitative traits (both vegetative and reproductive characters) was observed in aneuploids as compared to diploids. The present results are comparable to earlier observations pertaining to many other flowering plant species such as *Cassia demidiata* , *C. mimosoides* var. *wallichiana* and *Caesalpinia decapetala* [20], *Bupleurum lanceolatum*[21],*Epilobium angustifolium* and *Sium latijugam*[22] (Jeelani et al. 2017), *Oxalis corymbosa* [23], *Dioscorea deltoidea*[24].

Table 4. Detailed morphological comparison of diploid and Aneuploid cytotypes of *Pimpinella acuminata* Clarke.

S. No.	Characters	Dipliod cytotype (2n=18)	Aneuploid Cytotype (2n=20)
1.	Plant height (cm)	39.55- 43.80	67.51-73.20
2.	Number of Branches/ Plant	3-7	6-11
3.	Number of leaves/ plant	4-8	11-15
4.	Size of leaf (cm)	4.67-3.07×2.78-1.09	5.07-5.84×4.08-4.78
5.	Average stomata size (*µ*m)	9.64×5.08/ 13.05×11.40	21.14×17.89/ 25.18×21.15
6.	Average stomatal index of upper/ lower surface of leaf	61.57/30.78	64.86**/4**7.15

Each value based on minimum of 10 observations.

Meiotic Abnormalities:

The series of cytomixis, chromosomal tackiness, unoriented bivalents, laggards and bridges among the presently studied three species and ten populations level point towards the subsistence of intraspecific genetic diversity. The cytogenetical action of the chromatin conveys observed here were very similar to those in wild populations of Himalayan poppy (*Meconopsis aculeata* Royle) [25]. Such cytogenetic differences have also been reported in different plant species [26]. Cytomixis and chromatin stickiness are well proposed to be consequential from genetic factors [27] or natural factorsin addition to genomic–environmental line[28], and seems to be reliablyappropriate to the presently investigated taxa. The incidence of multipolar cells and meiotic irregularities in anaphase severance of chromosomes may be the reasonable mechanisms for the configuration of large-sized pollen grains and low pollen fertility in these meiotically abnormal populations as has been reported previously in quite a lot of angiosperms [29]. The hypo- and hyperploid PMCs are produceddue to cytomixis [27] (Fadaei *et al.* 2010) when accompanied with other meiotic abnormalities and mightdirect to uneven microsporogenesis substantial in the creation of variable-sized pollen grains perhaps with the aneuploid condition [27,28,29]. The occurrence of the flawedinconsistency of chromosome numbersbut enormousvariety in meiotic behavior at the intraspecificlevel of presently worked out three species demanding the necessitatefor the broad cytological exploration at population basison members of *Pimpinella* from different areas of north-westernHimalayas.

Ethnobotny:

*Pimpinella achilleifolia*commonly called "Anise"and whole plant is used for various ethnobotanical purposes. For the tribal peoples of the district Kangra, the whole plant crushed with mustard oil is a home remedy for abdominal discomfort and diarrhoea in domestic animal. In district Sirmour (HP), paste of leaves apply on wound, pimple and acne.

*P. acuminate*locally called as"Sodhf" and seeds are used for treatment of various diseases in tribes of North-West Himalayas. In District Kangra, the leaves and seeds are used to cure many digestive problems and wound healing, in domestic animals. Took 3-4 leaves of *P. acuminate* and ajwain seeds cursed with warm water and mixed with wheat flour then eat to animals to relief stomach pain and other digestive problems. Common Name of *P. diversifolia* "Jangli souf". Whole plant and leaves are used in various parts of District Kangra and Sirmour of Himachal Pradesh.In district Kangra, leaves and seeds are used as flavouring agent in many sweet dishes and also used to improve taste and smell of the dish. In district Sirmour, decoction of leaves and seeds used to cure indigestion and gastric problem

Traditionally, genus ***Pimpinella*** **L.** has several medicinal uses. In Ayurvedic medicine, it isused for liver and digestive problems. The plant is well known for its medicinal value as a good appetizerand as a remover of gastric, digestive problem, Liver problems and cough & cold[30]. *P. diversifolia* plant extract is used to cure cough and cold problems and digestive disorders in Garhwal Himalaya, India [31]. Locals of thePakistan use*Pimpinella acuminate*against gastrointestinal ailments, liver problems. The traditional uses of*Pimpinella acuminate* and ***P. diversifolia*** has not been reported previously.

CONCLUSION:

The present study concludes that meiotic abnormalities, particularly impaired male meiosis, are prevalent in *P. achilleifolia, P. diversifolia,* and certain populations of *P. acuminata* in the north-western Himalayas. The observed abnormalities in chromosome behaviour and pollen characteristics suggest potential challenges for the reproductive success of these species. The identification of a new diploid cytotype in *P. acuminata* contributes valuable information to the global cytogenetic knowledge of the genus *Pimpinella*.

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