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Understanding the status and epidemiological studies of downy mildew in Knol-khol across sub-tropical region of India

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Abstract
Knol-khol (*Brassica oleracea* var. *gongylodes* L.) is a cool-season crop widely cultivated in Jammu and Kashmir, West Bengal, and Karnataka. This study aimed to explore various aspects of downy mildew in Knol-khol, assessing disease status, and epidemiological studies. Symptoms on Knol-khol manifested as small angular spots with a pale yellow color, later turning black. On the lower side of the leaves, affected areas were covered with a grey-white downy growth containing numerous conidiophores bearing conidia. Morphological characteristics of *Peronospora parasitica* showed aseptate, hyaline conidia, and globose to spherical in shape, with an average length and breadth of 22.99 µm and 20.67 µm, respectively. Survey results in the Jammu division indicated considerable variation in disease incidence and intensity across locations in districts, Jammu and Samba, on different days after transplanting (DAT) - 30, 40, 50, and 60. Overall mean incidence and intensity recorded during these DAT periods were 12.66%, 9.73%; 21.77%, 17.48%; 41.07%, 36.02%; and 53.15%, 43.67%, respectively. The disease was significantly influenced by weather conditions during the cropping period, with a significant variation of 99%. Correlation data between weather parameters and disease intensity revealed highly significant positive correlations of 0.94 and 0.84 with the disease index for maximum and minimum temperatures. A highly significant negative correlation of -0.83 and -0.72 was observed with maximum and minimum relative humidity, respectively. However, a non-significant and negative correlation of -0.49 was noted with rainfall.

Keywords: Disease incidence, disease intensity, epidemiological studies, knol-khol, *Peronospora parasitica*

Introduction
Knol-khol (*Brassica oleracea* var. *gongylodes* L.) is a cool-season crop grown for its edible knob, also known as Khol rabi in India. It is an important cruciferous vegetable in Jammu and Kashmir, West Bengal, Maharashtra, Assam, Uttar Pradesh, Punjab, Odisha, and other parts of South India (Mishra *et al*., 2012) [10]. It is regarded for its short duration and hardiness, with a cultivated area of 2712 hectares and a production of 55118 metric tonnes in Jammu province (Bhushan *et al*., 2020) [3]. The fleshy knob, a stem growth above ground, is high in vitamins A and C, folic acid, dietary fibre, and minerals such as copper, calcium, potassium, manganese, iron, and phosphorus (Kumar *et al*., 2018) [5]. Knol-khol also has therapeutic characteristics that help with acidosis, asthma, cholesterol levels, cardiac problems, indigestion, and weight loss (Kumar *et al*., 2018) [5]. Jammu and Kashmir's distinct agro-climatic conditions favour Knol-khol growing. However, cool and wet weather during the crop's growth cycle causes a variety of biotic and abiotic stressors (Meziadi *et al*., 2016; Martins *et al*., 2018) [9, 6]. Biotic factors such as black rot, leaf spots, soft rot, and downy mildew pose serious concerns (Nyirenda *et al*., 2011) [12]. Downy mildew produced by Peronospora parasitica is especially damaging, causing production losses of 60-70% and impairing produce quality (Spencer, 1981) [17]. Small angular spots appear on immature leaves, evolving to irregular dark brown patches. In advanced stages, older leaves have papery, tan-colored lesions with grey-white downy growth on the lower side. Weather conditions are critical in the development of downy mildew. Banerjee *et al*. (2010) [2] discovered that the incidence is highest at temperatures ranging from 26 to 29 °C.

Achar (1998) [19] discovered a reduction in infection percentage at temperatures ranging from 15 to 26-30 °C. Understanding how environmental elements influence disease development can help farmers determine the best time to plant crops to reduce disease pressure (Saharan *et al*., 1997) [15].

Materials and Methods

The field experiments for the study titled "Understanding the Status and Epidemiological Studies of Downy Mildew in Knol-khol across Jammu and Samba Districts" were carried out at the Research Farm field of the Advance Centre for Rainfed Agriculture (ACRA) in Dhiansar, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, during the main cropping season of 2020-21. A systematic survey was conducted in the Jammu and Samba districts of the Jammu division in the Union Territory of Jammu and Kashmir from the first week of January to the first week of March 2021 to assess downy mildew disease affecting Knol-khol. Two tehsils/blocks were carefully chosen in each district, namely Jammu and RS Pura, and Samba and Vijay Pur. Five major Knol-khol growing villages were identified from each tehsil/block, and these villages, five major Knol-khol growing villages (Makwal, JwalaPur, ChakMalikah, Chak Bindrali and Lachman Pur) from Jammu Teshil, (Kheper, Rakh Nihalpur, Malik Pur, Kirpind and Kharian) from RS Pura Teshil, (Pangdhor, Sadoh, Mandi Kehli, Balouri and Chackjangi) from Samba Tehsil and (Badhori, Dagore, Chack Bathal, Sangwal, and Amwal) from Vijay Pur Tehsil, were identified. Five commercially significant Knol-khol fields were chosen at random. At 10-day intervals, disease incidence and intensity were rigorously recorded, with twenty-five plant samples randomly observed in each field. Symptomatology was also performed on Knol-khol plants exhibiting typical downy mildew symptoms. Plant pathology samples obtained during the survey were brought to the Plant Pathology Department's laboratory at Chatha, SKUAST Jammu. Symptomatic plants were grown in the field under natural settings to track the development of symptoms such as leaf spot, grey-white downy growth, and vascular discoloration. Conidial spores were gathered from infected leaves and multiplied on potted plants in the greenhouse before being sprayed on Knol-khol variety Large Green leaf plants. The development of typical downy mildew disease symptoms was monitored regularly, with one potted plant kept as a control without spore suspension spray. Under a microscope, morphological properties of Peronospora parasitica, such as the form, size, and colour of conidiophores, conidia, and sporangia isolated from Knol-khol leaves displaying typical symptoms, were detected. Morphological research was carried out using descriptions from numerous monographs and literature. Diseased leaf and plant samples gathered during the study were brought to the plant pathology department's laboratory at Chatha SKUAST Jammu. Symptomatic plants were grown in natural conditions to track the development of symptoms such as leaf spots, grey-white downy growth, and vascular discoloration. Conidial spores were collected from infected leaves, replicated in potted plants under greenhouse conditions, then sprayed on Knol-khol variety "Large Green" plants. The development of downy mildew symptoms on inoculated plants was monitored regularly. Under a microscope, the morphological properties of Peronospora parasitica were investigated, including the shape, size, and colour of conidiophores, conidia, and sporangia isolated from Knol-khol leaves. 900 Knol-khol plants were used in the field experiment at ACRA Dhiansar's Research Farm during the main cropping season of 2020-21. Plants were inspected for disease development at weekly intervals from transplanting to crop harvest, with disease intensity recorded during observations. Concurrently, meteorological data on temperature, relative humidity, and rainfall were gathered from the Dhiansar meteorological research station, and correlation and regression analyses were performed between illness intensity and meteorological parameters.

Results and Discussion

The initial signs of downy mildew sickness in Knol-khol were little, angular, pale yellow spots that eventually turned black on the plants' young leaves. The lesions merge to form amorphous, dark brown regions on both surfaces of the leaves as they advance. The injured portions on the underside of the leaves are surrounded by a downy, grey-white growth composed of many conidiophores bearing conidia. Young damaged leaves typically fall off the plant as they become yellow, while mature leaves remain on the plant and exhibit the pathogen's unique downy growth. As the disease spreads on older leaves, the lesions become papery and brown, making them readily ripped. Furthermore, a longitudinal cut reveals a greyish-black discolouration within the leaf stem. Due to severe illnesses, entire leaves perish. Natti *et al*. (1956) [11] and Srivastava *et al*. (2014) [18], who saw similar symptoms on the Knol-khol, corroborate these findings. Kolte (1985) [4], Sherf and Macnab (1986) [16], and Saharan (1992) [14] studies all support our findings.

Conidial spores were removed from the diseased leaves and cultivated on potted plants in a greenhouse. The conidial suspension from the extended inoculum was used to assess the pathogenicity of the Peronospora parasitica pathogen on the knol-khol plant. Young leaves show the first signs of the disease one week after inoculation. They can be identified by a downy, discoloured growth on the lower side and pale yellow, angular spots scattered across the upper surface. The pathogen causes as many conidiophores with conidia to sporulate beneath the infected leaves' lower surface patches. However, in the later stages of the illness, the lesions on older leaves take on a papery, tan tone that renders them readily ripped. In addition, a greyish-black discoloration is visible on a leaf stem that has been cut longitudinally.

The shape and size of the conidiophore and conidia recovered from Knol-khol infected leaves, as well as other morphological aspects of the Peronospora parasitica pathogen, were evaluated under a microscope utilising Table 1. The hyaline-colored conidiophores had a flattened base and a robust main axis, and they were homogenous in size, measuring between 189.23 and 288.76 µm. These conidiophores had six to eight dichotomously branched terminals that sharply separated and considerably thickened above each fork. The terminal branches ended in a single conidium and were long, thin, and pointed. In the beginning, the juvenile conidia were spherical, hyaline, and aseptate. When the conidia reached maturity, they were globose to spherical in shape, averaging 22.99 µm in length and 20.67 µm in breadth. Their lengths ranged from 19.69 to 28.66 µm and their widths from 17.09 to 23.10 µm. The physical properties of Peronospora parasitica corroborated the conclusions provided by Mehta and Saharan (1994) [7].

During the winter season of 2020–21, a systematic study was conducted in the key Knol-khol growing areas of Jammu and Samba districts to analyse disease incidence and intensity in order to determine the current state of downy mildew disease in Knol-khol in the Jammu division of UT Jammu and Kashmir. For the survey, observations were taken at 30, 40, 50, and 60 days after transplanting (DAT). Significant variation in sickness incidence at several DAT points (i.e., 30, 40, 50, and 60 days) across all locations in the two districts was evident from the examination of Table 2 and Fig-1 data. The mean incidence of disease over these periods was 12.66%, 21.77%, 41.07%, and 53.15% in the districts that were the subject of the study. Surprisingly, district Samba's Sadoh had the highest disease incidence (16.46%) at 30 DAT, while district Samba's Badhori had the lowest (7.65%). Sadoh (district Samba) had the highest incidence (25.49%) and Lachman Pur (district Jammu) had the lowest (16.84%) when 40 DAT were compared. Sangwal (district Samba) had the lowest disease incidence at 50 DAT, while Makwal (district Jammu) had the highest at 52.62%. As we advanced to 60 DAT, the disease incidence in Mandi Kehli (district Samba) was 44.63%, while the highest was 63.19% in Kirpind (district Jammu). In the 30, 40, 50, and 60 days following transplantation (DAT), district Samba had the lowest incidence of downy mildew (12.43%), whereas district Jammu had the highest incidence (12.58%, 44.49%, 57.85%, and 22.58%, respectively). Jammu and Samba were the two districts from which these statistics were collected..

As shown in Table 3, there was a considerable variance in disease intensity at each of the two districts' sites and at various days after transplanting (DAT)—30, 40, 50, and 60. The mean overall sickness intensity in the districts under investigation was 9.73%, 17.48%, 36.02%, and 43.67%, in that order. At 30 DAT, Sadoh (district Samba) had the highest disease intensity (12.46%), while ChackJangi (district Samba) had the lowest disease intensity (5.56%). Rakh Nihalpur (district Jammu) had the highest intensity (21.84%) at 40 DAT, while Amwal (district Samba) had the lowest (14.18%). At 50 DAT, Badhori (district Samba) had the lowest illness intensity (25.55%), whereas Chak Malikhan (district Jammu) had the highest (48.07%). As we approached 60 DAT, Chak Malikhan (district Jammu) had the highest level of sickness intensity (49.40%), while Dagore (district Samba) had the lowest (36.22%). The study discovered that the maximum severity of downy mildew was reported in Jammu district (9.67%, 18.05%, 41.43%, and 46.03%, respectively) and Samba district (9.53%, 16.76%, 31.32%, and 41.62%, respectively) at 30, 40, 50, and 60 days after transplanting (DAT). Our findings are very close to the findings of Saharan (1984) [13].

A field experiment was conducted at ACRA Dhiansar in the Samba district to investigate the downy mildew disease intensity in Knol-khol and the related weather conditions during the cropping period. Crop sowing dates under low disease pressure can be altered by studying how weather conditions (meteorological factors) influence disease development. Table 4 and Figure 3 show disease intensity data as well as meteorological data from the first to tenth meteorological standard week, including maximum and minimum temperatures, relative humidity, rainfall, and maximum and minimum humidity. The findings revealed that the disease grew slowly during the first normal meteorological week, with small spots forming on the leaves, and that the disease intensity was 0% during this week. During the second meteorological week, illness intensity was 11.34 percent, with zero rainfall, maximum and minimum temperatures of 14.90 and 8.70 °C, and maximum and minimum relative humidity of 94 and 80 percent. Despite a constant increase in illness intensity, the percentage rate of rise was not consistent from the third to the sixth meteorological week due to a reduction in the lowest temperature. Beginning in the seventh meteorological week, there was a significant increase in the intensity and rapidity of disease progression. Higher maximum temperatures (21.5-28.6 °C) and lower relative humidity (96-80%) were associated with these increases. The tenth meteorological week saw maximum and minimum temperatures of 28.6 and 11.7 °C, respectively, as well as maximum and minimum relative humidity of 80 and 39 percent, with a maximum illness intensity of 63.76 percent. These findings are supported by the research of Sinobas Alonso and Diaz Alonso (1995) [1], Mehta *et al*. (1995) [8], and Saharan *et al*. (1997) [15], who discovered that a temperature of 15 °C or higher appears to be the most favourable development of an epidemic because it promotes slower growth of the pathogen and host, resulting in less severe damage.

The correlation data in Table 5 show that rainfall, minimum and maximum temperatures, relative humidity, and humidity levels all have a substantial impact on the disease's development. The intensity of downy mildew in Knol-khol was shown to be extremely significant and positively related to maximum and lowest temperatures, with values of 0.938 and 0.840, respectively. Furthermore, a substantial negative relationship was discovered between the percentage of disease intensity and the maximum and lowest relative humidity, with correlation values of -0.83 and -0.72, respectively. In contrast, there was a 0.49 non-significant negative correlation between illness intensity and rainfall. Based on meteorological data such as maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity, and rainfall, regression analysis (Table 6) revealed a 99 percent significant fluctuation in the growth of downy mildew in Knol-khol. Our findings are supported by the findings of Banerjee *et al*. (2010) [2], who discovered that the maximum temperature was positively correlated with the downy mildew disease index and that a maximum temperature between 26 and 29 °C and a relative humidity of more than 65% favours the development of downy mildew disease.

Table 1: Morphological characteristics of *Peronospora parasitica*

Morphological stage	Color	Shape	Size	Septation
Conidiophore	Hyaline	Uniform flattened base and stout main axis and dichotomously branched (6 to 8 times), tips are bifurcate and branch at an acute angle	189.23–288.76 µm	Aseptate
Conidia	Hyaline	Globose to spherical	19.69-28.66×17.09-23.10 µm (22.99 × 20.67 µm)	Aseptate

*Values are mean of 15 replications

****** Values in parenthesis are mean values

Table 2: Percent incidence of downy mildew of Knol-khol at various locations of Jammu and Samba districts during 2020-21

District	Tehsil/Block	Villages	Disease Incidence			
			30DAT	40 DAT	50 DAT	60 DAT
Jammu	Jammu	Makwal	9.62	19.25	52.62	59.82
		ChakMalikhan	12.08	20.82	51.69	60.89
		JwalaPur	9.45	17.48	42.82	57.19
		ChakBindrali	11.69	18.21	47.82	63.19
		Lachman Pur	10.23	16.84	45.29	61.24
	RS Pura	Kheper	11.3	24.00	52.62	59.82
		RakhNihalpur	15.32	23.21	51.69	60.89
		Malik Pur	9.89	22.34	42.82	57.19
		Kirpind	13.26	25.02	47.82	63.19
		Kharian	12.36	19.00	45.29	61.24
	Mean±S.E		13.00±0.69	22.58±0.86	44.49±1.38	57.85±1.31
	Range		9.45-15.32	16.84-25.02	42.82-52.62	57.19-63.19
	C.I (p<0.05)		11.39-13.70	20.59-23.45	41.95-48.66	54.83-59.17
Samba	Samba	Pangdhor	12.98	23.86	37.29	48.32
		Sadoh	16.46	25.49	38.29	52.81
		Mandi Kehli	10.29	21.82	35.21	44.63
		Balouri	13.69	22.46	40.15	46.21
		Chackjangi	9.56	19.65	34.86	47.23
	Vijay Pur	Badhori	7.65	15.45	35.89	50.44
		Dagore	13.87	21.76	36.18	46.22
		ChackBathal	14.89	20.87	39.5	49.65
		Sangwal	13.6	19.32	32.17	51.39
		Amwal	11.9	17.55	38.92	45.52
Mean±S.E			12.43±0.93	20.49±0.98	36.79±0.86	48.23±0.97
Range			7.65-16.46	15.45-25.49	32.17-40.15	44.63-52.81
C.I (p<0.05)			10.27-14.60	18.24-22.74	34.80-38.80	46.00-50.46
Over all Mean			12.66	21.77	41.07	53.15

Table 3: Percent disease intensity of downy mildew of Knol-khol at various location of Jammu and Samba districts during 2020-21

District	Tehsil/Block	Villages	Disease Intensity			
			30DAT	40 DAT	50 DAT	60 DAT
Jammu	Jammu	Makwal	8.49	18.05	41.94	46.42
		ChakMalikhan	10.55	19.66	48.07	49.40
		JwalaPur	8.46	16.36	39.82	45.00
		ChakBindrali	10.36	16.96	44.47	47.83
		Lachman Pur	8.99	15.89	42.12	48.54
	RS Pura	Kheper	7.73	16.77	42.47	41.02
		RakhNihalpur	11.57	21.84	37.31	48.32
		Malik Pur	10.11	17.94	35.63	47.01
		Kirpind	8.70	17.42	43.69	42.16
		Kharian	11.71	19.57	38.80	44.61
	Mean±S.E		9.67±0.43	18.05±0.58	41.43±1.15	46.03±0.88
	Range		7.73-11.71	15.89-21.84	35.63-48.07	41.02-49.40
	C.I (p<0.05)		8.68-10.66	16.74-19.35	38.81-44.05	44.03-48.03
Samba	Samba	Pangdhor	10.98	18.86	30.29	41.32
		Sadoh	12.46	20.49	33.29	45.81
		Mandi Kehli	8.23	17.82	30.21	39.63
		Balouri	11.69	19.46	37.15	41.21
		Chackjangi	5.56	16.65	27.86	47.23
	Vijay Pur	Badhori	5.88	14.3	25.55	43.44
		Dagore	10.66	16.89	30.78	36.22
		ChackBathal	12.24	15.26	34.23	39.15
		Sangwal	10.89	15.81	27.67	42.39
		Amwal	8.16	14.18	35.1	39.52
Mean±S.E			9.53±0.88	16.76±0.72	31.32±1.29	41.62±1.15
Range			5.56-12.46	14.18-20.49	25.55-37.15	36.22-47.23
C.I (p<0.05)			7.49-11.57	15.08-18.44	28.33-34.30	38.94-44.29
Over all Mean			9.73	17.48	36.02	43.67

Table 4: Effect of weather components on the development of downy mildew disease of Knol-khol

STM	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	Disease intensity (%)	Per cent increases in diseases
	Max	Min	Max	Min			
1.	18.4	1.6	96	62	12.6	0	0
2.	14.9	8.7	94	80	0.0	11.34	11.34
3.	17.9	6	95	73	0.0	15.72	4.38
4.	17.8	5	92	63	0.5	17.063	1.343
5.	19.8	6	89	50	0.0	20.78	3.717
6.	21.1	6.9	92	53	0.0	22.06	1.28
7.	21.5	10.4	96	68	0.0	28.45	6.39
8.	25.9	10.5	92	50	0.0	46.45	18
9.	26.5	10.4	84	50	0.0	55.6	9.15
10.	28.6	11.7	80	39	0.0	63.76	8.16

Table 5: Correlation coefficients between the weather parameters and downy mildew disease of Knol-khol

S. No	Weather parameters	Downy mildew
1.	Maximum temperature	0.94^{**}
2.	Minimum temperature	0.84^{**}
3.	Maximum relative humidity (%)	-0.83^{**}
4.	Minimum relative humidity (%)	-0.72^{**}
5.	Rainfall (mm)	-0.49^{NS}

Table 6: Linear regression of epidemiological factors with the disease index of downy mildew disease of Knol-khol

Variety	Linear regression	Correlation coefficient (R)	Coefficient of determination (R^2)
Large green	Y=23.744+3.905X1+0.544X2-1.184X3+0.442X4+0.806X5	0.997	0.994

Where,
X1 = Maximum temperature (°C)
X2 = Minimum temperature (°C)
X3 = Maximum relative humidity (%)
X4 = Minimum relative humidity (%)
X5 = Rainfall (mm)
Y = Disease Incidence (%)

Conclusion
In conclusion, knowledge about Jammu's Knol-khol downy mildew was gained through fieldwork conducted at ACRA. The research validated the course of the illness, confirming earlier findings. The pathogenicity of Peronospora parasitica was shown by inoculation, which is consistent with previous research. Most importantly, the study revealed the significant influence of weather conditions, with temperature and humidity affecting the severity of the illness. This association was statistically supported by correlation and regression analysis. The results have practical implications for farmers, providing useful insights for modifying sowing intervals and putting into practice efficient disease management plans, which will ultimately support the region's sustainable cultivation of Knol-khol.

Future of Scope
The results of the ACRA field studies on Knol-khol downy mildew in Jammu offer a starting point for disease control efforts in this crop going forward. The dynamics of the disease are better understood in light of the confirmed course of the illness and the proven pathogenicity of Peronospora parasitica. The importance of taking climate variables into account in disease control methods is suggested by the prominent influence of meteorological parameters. This realisation has potential applications in the creation of personalised therapies and predictive models. Regression and correlation analyses provide statistical information that provides a quantitative foundation for decision-making. The implementation of targeted control methods and adjustment of sowing schedules are among the practical consequences for farmers that hold significant importance in ensuring the sustainability of Knol-khol agriculture in the region going forward. These results open the door for knowledgeable and flexible approaches to guarantee the resistance of Knol-khol crops against downy mildew as long as agricultural difficulties exist.

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Conflict of interest: None.

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