Int. J Seed Spice, 13 (1 & 2), January & July, 2023: 34-43 DOI: https://doi.org/10.56093/IJSS.v13i1-2.5

Reviewed by
Dr. Sumer Singh Puniya
Sri Karan Narendra Agriculture University, Jobner,
Email: sspunia.pbg@sknau.ac.in

***Correspondence**
R.D. Meena
meenard2005@gmail.com

Received: 06 August 2024
Revision: 24 September 2024
Accepted: 11 November 2024

Citation
Meena,S.S., Meena, R.D.*, Lal, G., Malhotra, S.K., Meena, R.S., Meena, M.D., Chaudhary, N., Saxena, S.N., Tripathi, K. and Choudhary, R. 2023. AJMER NIGELLA-1: Pioneering High-Yield Nigella Variety, *Int. J Seed Spice,* 13(1&2): 34-43

DOI
https://doi.org/10.56093/IJSS.v13i1-2.5

Affiliation
[1]ICAR-National Research Centre on Seed Spices, Ajmer (Rajasthan),

Ajmer Nigella-1: Pioneering High-Yield Nigella Variety

S.S. Meena, R.D. Meena*, G. Lal, S.K. Malhotra, R.S. Meena, M.D. Meena, N. Chaudhary, S.N. Saxena, K. Tripathi, and R. Choudhary

Abstract
A comprehensive assessment was undertaken to identify high-yielding *Nigella sativa* genotypes. Analysis of diverse germplasm revealed significant phenotypic variation in key agronomic traits, with seed number per capsule, days to maturity, and seed yield per plant exhibiting the broadest ranges. The yield performance of nigella variety AN-1 (1129.36 kg/ha) (10.20% higher than Ajmer Nigella-20) was found superior for seed yield and other yield attributing characters then check varieties AN-20 and Pant Krishna. Total oil content in AN-1 was recorded 19.70% (37.76 and 33.10 % higher than Ajmer Nigella-20 and Pant Krishna, respectively**)** whereas essential oil content was 0.03 %. The proposed variety of Ajmer Nigella-1 (AN–1) overall performance was significant. Out of 54 primers used in molecular analysis only 5 primers showed Polymorphism.

Keywords: Nigella, variability, quality attributes, range, polymorphism.

Introduction
Nigella (*Nigella sativa* L.), a member of the Ranunculaceae family, is a seed spice crop of notable medicinal importance. This crop is predominantly cultivated in various Indian states, including Madhya Pradesh, Bihar, Punjab, and Assam. Beyond India, Nigella is extensively grown in countries such as Pakistan, Sri Lanka, Bangladesh, Nepal, Egypt, and Iraq. Recognized as one of the most widely utilized medicinal plants globally, Nigella's seeds contain essential oil with concentrations ranging from 0.5% to 1.4%. This essential oil is highly valued in the perfume and medicinal industries for its diverse applications. (Malhotra, 2004, Dubey *et al.,* 2016). Rajasthan, renowned for its cultivation of diverse seed spices, is witnessing a burgeoning interest in Nigella sativa as a potential alternative crop. The state's arid climate, particularly in the southeastern region, has proven conducive to Nigella cultivation. With its high market demand and potential for economic returns, Nigella is emerging as a promising addition to Rajasthan's

agricultural landscape. (Preeti *et al*., 2019). Nigella cultivation has shown significant growth in Rajasthan during the 2015-16 agricultural season. The state cultivated a total of 10,157 hectares of Nigella, with Jhalawar district emerging as the leading producer, accounting for 7,167 hectares of the total area.

In terms of production, Rajasthan yielded a total of 8,522 metric tons (MT) of Nigella, with Jhalawar district again taking the forefront, contributing 5,734 MT to the state's total output. Chittor district followed closely behind Jhalawar in both area and production. (Preeti *et al*., 2019). The extracts of Nigella seeds have been used by patients to suppress coughs (Mahfouz *et al*., 1960), disintegrate renal calculi (Hashem and El-Kiey, 1982), retard the carcinogenic process (Aftab *et al*., 2013; Hassan and El-Dakhakhny, 1992; Worthen *et al*., 1998), treat abdominal pain, diarrhea, flatulence and polio (Enomoto *et al*., 2001), exert choleretic and uricosuric activities (El-Dakhakhny, 1965), anti-inflammatory (Chakravarty, 1993; Houghton *et al*., 1995) and antioxidant effects (Mansour *et al*., 2002).

Materials and methods

To ensure a healthy crop, all recommended agricultural practices were followed. The experimental design was a Randomized Block Design (RBD) with a row-to-row distance of 10 cm and a plant-to-plant distance of 25 cm, and a plot size of 2.5x4.0 m^2. Alongside AN-1, two check varieties, Ajmer Nigella-20 (AN-20) and Pant Krishna (PK), were also grown. Observations were recorded for various growth and yield parameters as follows:

The oil extraction methodology for Nigella seeds was carried out using the Hydro Distillation (HD) method as described by Kokoska et al. (2008). Initially, the Nigella seeds were ground at a controlled temperature of 25°C. A 70 g sample of the ground seeds was then weighed for subsequent analysis. The extraction process employed a Clevenger-type apparatus, characterized by its water-holding flask directly connected to a condenser. The prepared sample was placed in the flask, and the distillation process was conducted continuously for 2 hours. Upon completion of the distillation, a pale-yellow essential oil was obtained. The average yields were calculated based on the dry weight of the sample. This method provided an efficient approach to extracting essential oil from Nigella seeds, ensuring the purity and quality of the final product.

DNA extraction, purification, quantification

DNA extraction from seed samples of the candidate variety Ajmer Nigella-1 and its closely resembling variety Ajmer Nigella-20 was performed using a modified CTAB method. The quantity and quality of the extracted DNA were assessed using 0.8% agarose gel electrophoresis and Eppendorf Biophotometer readings. Only high-quality DNA was selected for subsequent ISSR (Inter-Simple Sequence Repeat) analysis.

Analysis

The PCR reaction was conducted using 10-20 ng of DNA as the template and Emerald Green PCR Mastermix (Takara). The PCR products were analyzed on 2% agarose gels, which were run at 80V for 2-2.5 hours and visualized using a UV transilluminator. Data was scored based on the presence (1) or absence (0) of bands. A total of 54 primers were used in the analysis. Among these, polymorphic primers and the total alleles observed with primer Sola 2 revealed five distinct alleles.

Results and discussion

The significant differences among all the genotypes for most of the traits recorded; indicating the presence of significant variation in the material there by justifying the selection of the experimental material. Highest seed yield recorded in variety AN-1 (1129.36 kg/ha) followed by AN-20 (1024.81kg/ha) and Pant Krishna (760.31 kg/ha) (Table 1). The range and mean of AN-1 for various characters are presented (Table 2). The range of characters indicated the existence of variability for all the characters. The range of variation was high for seeds/capsule (70.3-97.2) followed by days to maturity (145-150) and seed yield/plant (g) (59.2-63.3). The similar results were found by Preeti *et al*. (2019). Table 3 showed that quality attributes of variety AN-1 contained total oil 19.70 % and essential oil 0.03%, it was greater than Ajmer Nigella-20 (37.76 %) and Pant Krishna (33.10 %)**.** Similar studies related to the oil extraction from the nigella were done earlier by Salma *et al*. (2007) and Farhan *et al*. (2021). **Figure 1** indicated profiling of total oil extracted from seeds of AN-1 showed that Cis-11,14- Eicosadienoic Acid.

Eicosadienoic Acid % (3.145) was found maximum in AN-1 compared to both the check varieties. **Figure 2** illustrated root rot disease intensity in AN-1 was recorded (PDI 4.4) less than both the check varieties

namely AN-20 (PDI 6.2) and Pant Krishna (PDI 8.0). The most suitable sowing date was recorded 15 October at which the variety produced maximum seed yield (821 kg/ha) and maximum test weight (2.39g) (Table 4). Almost similar results about test weight (weight of 1000 Nigella seeds) were found in research earlier conducted by Getachew and Beriso (2020). Effect of sowing dates, NPK levels and weedicides on growth parameters at different growth stages of AN-1 indicated high seed yield at sowing date 1st November (Table 5 and Table 6)**.** Performance of proposed variety of Ajmer Nigella-1 (AN-1) was tested by conducting demonstrations at a farmer's field (**Figure 3**). Out of 54 primers used in PCR only 5 primers showed Polymorphism. 1 polymorphic primer (Sola 2) showed an amplification band on 650 bp having the Sequence (5'-3' DDCGACGACGACGACGA) (Table 7).

Conclusion:

Nigella is an important seed spice crop that exhibits considerable variability. The yield performance of the Nigella variety AN-1 was 1129.36 kg/ha, which is 10.20% higher than Ajmer Nigella-20, demonstrating its superiority over both check varieties (AN-20 and Pant Krishna). The total oil content in AN-1 was approximately 33% greater compared to the check varieties. Additionally, the intensity of root rot disease in

Table 1: Performance of proposed nigella variety AN-1 over a check at Ajmer in CVT during 2016-2019 (Pooled)

Entries	Seed Yield (kg/ha)	Rank as per seed yield
NDBC-20	732.00	9
NDBC-21	761.61	6
AN-1	1129.36	1
AN-20 (C)	1024.81	2
HKL-7	733.39	8
IN-1	703.67	10
AN-23	818.33	5
PK (C)	760.31	7
PK-2	927.67	4
PK-1	958.94	3
C.D. 5%	101.23	
SEm±	33.81	
C.V. %	6.85	

Table 2: Ancillary data for the proposed variety tested at Ajmer locations in CVT during 2016-17 to 2018-19

Characters	AN-1		AN-20 (NC)	P.K. (NC)
	Range	Mean		
Plant Height (cm)	66.2-70.2	68.4	67.4	69.3
Primary Branch/Plant	9.7-11.9	11.3	10.2	8.5
Secondary Branch/Plant	31.3-34.4	32.6	30.4	26.0
Days to 50%flowering	83-87.8	84.44	85.89	85.22
Days to maturity	145-150	147.5	150	149
No. of capsule/plant	65.6-69.6	69.4	55.1	51.3
Seeds/capsule	70.3-97.2	89.2	86.8	83.2
Seed yield/plant (g)	59.2-63.3	61.5	47.4	35.7
1000 Seed weight	2.0-2.4	2.2	2.1	2.1
Seed Yield (kg/ha)	1029.7-1201.6	1129.3	1024.8	760.3

Table 3: Quality attributes of the proposed variety AN-1 tested at NRCSS, Ajmer during 2017-18

Name of entries	Total Oil (%)	Essential Oil (%)
AN-1	19.70	0.03
Ajmer Nigella-20 (NC)	14.30	0.08
Pant Krishna (NC)	14.80	0.03
% higher than Ajmer Nigella-20	37.76	
% higher than Pant Krishna	33.10	

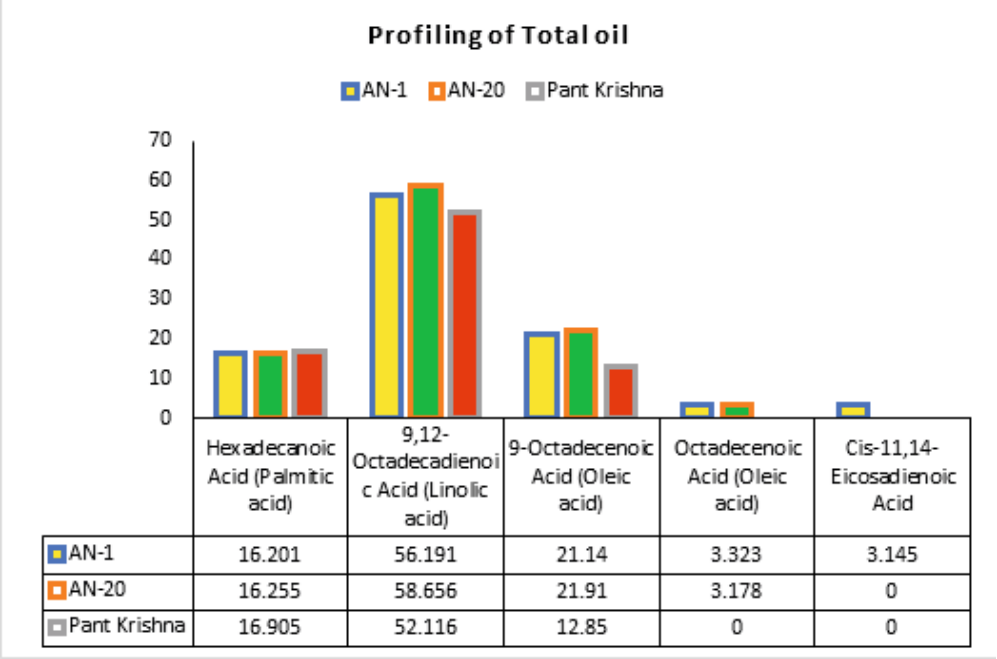

Figure 1: Profiling of Total oil extracted at Ajmer from seeds of AN-1during 2017-18

Table 4: Profiling of Total oil extracted at Ajmer from seeds of AN-1during 2017-18

Compounds (%)	AN-1	AN-20	Pant Krishna
Hexadecanoic Acid (Palmitic acid)	16.201	16.255	16.905
9,12- Octadecadienoic Acid (Linolic acid)	56.191	58.656	52.116
9-Octadecenoic Acid (Oleic acid)	21.14	21.91	12.85
Octadecenoic Acid (Oleic acid)	3.323	3.178	0
Cis-11,14- Eicosadienoic Acid	3.145	0	0

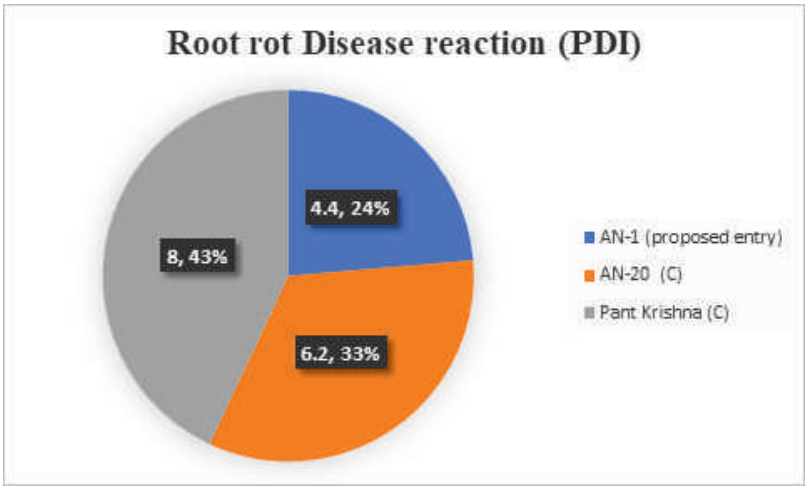

Figure 2: Disease reaction (PDI) observed of proposed variety AA-73 at ICAR-NRCSS, Ajmer during 2017-18

Table 4: Effect of sowing dates and crop geometry on growth, yield attributes and seed yield of AN-1during 2016-17

Treatments	Plant height (cm)	No. of capsule/ plant	No. of seeds/ capsule	Capsule weight (mg)	Thousand seed weight (g)	Seed yield (kg /ha)
Date of sowing						
1st October	51.13	33.07	80.16	25.34	2.06	590
15th October	60.47	51.76	92.67	27.21	2.39	821
30th October	57.27	46.29	84.09	26.92	2.20	591
15th November	43.36	26.42	77.47	18.08	1.95	155
30th November	29.22	13.31	65.69	15.01	1.77	107
SEm ±	0.73	1.52	2.04	1.11	0.08	0.44
CD (P=0.05)	2.12	4.41	5.92	3.20	0.23	1.27
Crop geometry						
Row to Row and plant to plant spacing 20*10 cm	48.16	32.87	76.55	22.77	2.01	418
Row to Row and plant to plant spacing 25*10 cm	48.52	36.23	83.17	21.15	2.12	488
Row to Row and plant to plant spacing 30*10 cm	48.19	33.41	80.32	23.62	2.10	453
SEm ±	0.57	1.78	1.58	0.86	0.06	0.34
CD (P=0.05)	1.64	3.41	4.59	2.48	0.17	0.98

Table 5: Effect of sowing dates, NPK levels and weedicides on growth parameters at different growth stages of AN-1 during 2017-18

Treatments	Plant height (cm)	No. of Primary branches/Plant	No. of Secondary branches/Plant	Days to 50% flowering
Sowing dates				
1st November	44.0	6.6	6.0	4.5
15th November	43.2	0.24	0.69	8.5
30th November	40.0	6.6	5.5	0.51
S.Em±	1.30	1.48	77.9	75.1
CD (p=0.05)	NS	71.1	0.18	0.53
NPK levels				
30:30:15 kg/ha	40.4	5.1	5.9	74.7
40:40:20 kg/ha	44.9	6.0	7.4	74.8
50:50:25 kg/ha	41.8	6.0	7.4	74.6
S.Em±	1.30	0.24	0.51	0.18
CD (p=0.05)	NS	0.69	NS	NS
Weedicides				
Pendimethalin @ 1.0 kg/ha	41.7	5.9	7.1	70.1
Oxadiargyl @ 0.75 kg/ha	43.0	5.6	6.7	18.0
S.Em±	1.06	0.19	0.42	8.68
CD (p=0.05)	NS	NS	NS	24.96

Table 6: Effect of sowing dates, NPK levels and weedicides on yield parameters and seed yield of AN-1during 2017-18

Treatments	Siliqua per plant	Seed per siliqua	Seed yield (kg/ha)	Straw yield (kg/ha)	Test weight (g)
Sowing dates					
1st November	16.1	71.1	778.8	1484.0	2.4
15th November	15.8	70.6	659.9	1249.2	2.2
30th November	11.4	66.4	328.6	1012.0	1.7
S.Em±	0.36	0.38	23.9	57.6	0.05
CD (p=0.05)	1.05	1.10	68.8	165.6	0.15
NPK levels					
30:30:15 kg/ha	12.8	66.4	490.7	1220.3	2.1
40:40:20 kg/ha	15.7	72.6	677.2	1367.2	2.2
50:50:25 kg/ha	14.9	69.2	599.6	1157.7	2.0
S.Em±	0.36	0.38	23.9	57.6	0.05
CD (p=0.05)	1.05	1.10	68.8	165.6	0.15
Weedicides					
Pendimethalin @ 1.0 kg/ha	13.1	66.9	544.8	1110.7	2.0
Oxadiargyl @ 0.75 kg/ha	15.8	71.9	633.5	1386.1	2.2
S.Em±	0.30	0.31	19.5	45.8	0.04
CD (p=0.05)	0.86	0.90	56.1	131.7	0.12

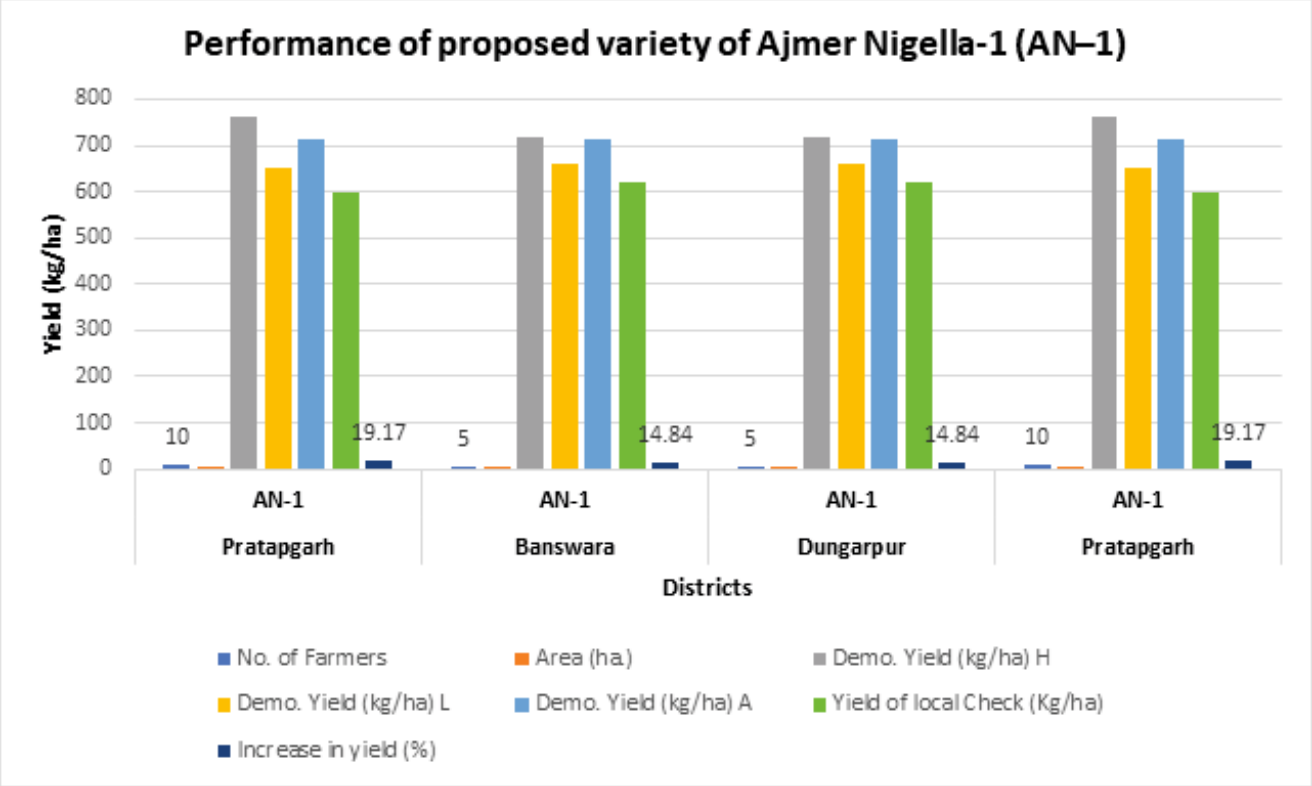

Figure 3: Performance of proposed variety of Ajmer Nigella-1 (AN–1) in demonstrations conducted by NRCSS Ajmer at farmer's field during 2017-18

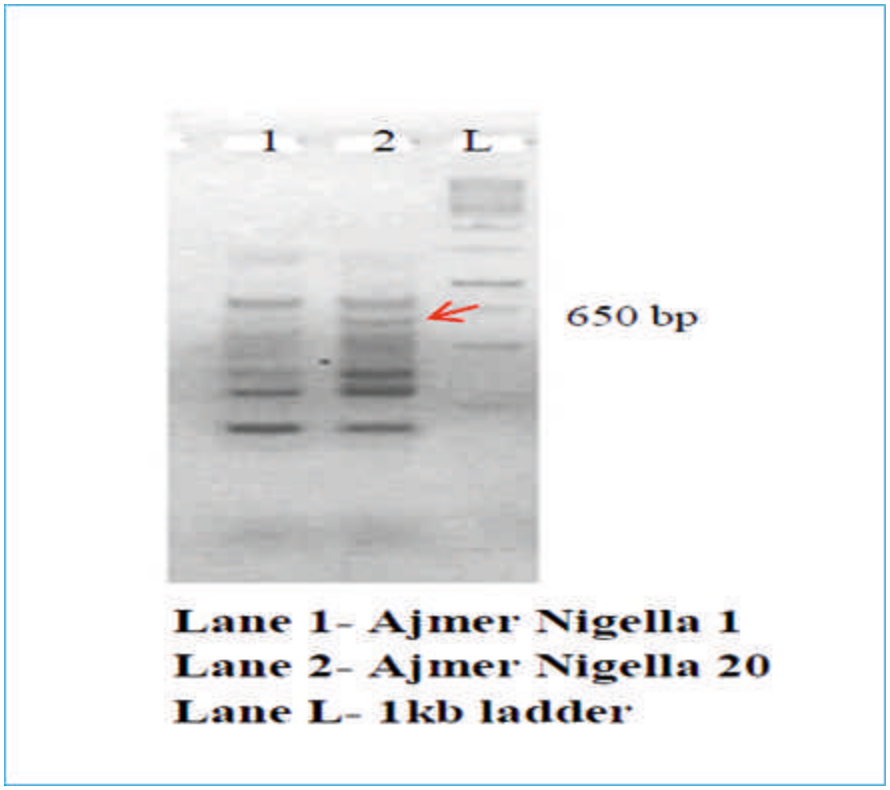

Table 7: Scorable bands observed and band size

Primer Name	Ajmer Nigella-01 (Candidate variety)	Ajmer Nigella-20 (Check variety)	Size (bp)
Sola 2	1	1	800
	0	1	*650
	1	1	350
	1	1	300
	1	1	100

(* The polymorphic band indicated in red distinguished Ajmer Nigella-1 from Ajmer Nigella-20.)

Figure 4: Morphological features of Ajmer Nigella-1 a) Primary stage, b) Flower C) Plant d) seed

AN-1 was recorded as being lower than in the check varieties. The overall performance of the proposed Ajmer Nigella-1 (AN-1) variety was found to be significant. Molecular analysis further indicated that the AN-1 variety is genetically distinct from the existing Nigella variety AN-20.

Conflicts of Interest: The authors declare no conflicts of interest.

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