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Varietal Screening of Radish Genotypes Against Alternaria Leaf Spot (*Alternaria raphani*) at Western Nepal

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ARTICLE INFO	A B S T R A C T
Research Article	Paucity of knowledge on resistant variety, high yield loss ranging from 32- 57% and dearth of knowledge on management of <i>Alternaria</i> leaf spot has been the major concerns in radish production in the world. Our study will aid in multiplication and standardization of the germane variety of radish
Received : 25/06/2022 Accepted : 17/11/2022	resistant to <i>Alternaria</i> leaf spot disease so as to escalate the production, productivity, and the competitiveness in the domestic as well as in the international market. Hence, this experiment was rendered to screen the radish genotypes against <i>Alternaria</i> leaf spot disease in Western Nepal in 2021. Seven genotypes namely Sparkle white F1 hybrid, Korean cross, Palak-patta 35, Pyuthane red, 40 days. Gente, 32, and Mine aerly used as the treatment and everyment use leid out in
<i>Keywords:</i> Radish <i>Alternaria raphanin</i> AUDPC Disease assessment Host response	red, 40 days, Geeta 33, and Mino early were used as the treatment and experiment was laid out in one factorial RCBD design. Each treatment was assigned with 8 plants for observation with 3 replications per treatment. Parameters like days to appearance of disease, incidence of disease on seedling, disease severity (3 scorings), mean score of disease, mean AUDPC and reaction of genotypes were measured during the experiment. Our study unveiled highly significant result for the days to appearance of disease, disease severity, mean score of disease and AUDPC among the genotypes of radish. <i>Alternaria</i> leaf spot first appeared in Pyuthane red (18.33 days) while appeared late in Mino early. Disease severity was found harsh in 40 days at each scoring (57.33, 64.67 and 70.33 respectively); on the contrary severity was minor in Sparkle white at each scoring (26.67, 35 and 44.17 respectively). Similarly, the highest MDS (3.4) and AUDPC (449.8) was found in 40 days, whereas the lowest MDS (1.7) and AUDPC (246.5) was observed in Sparkle white. Ultimately, it is contended that 40 days was found to be moderately susceptible variety while other varieties were found to be moderately resistant.
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Introduction

Radish Raphanus *raphanistrum* subsp. *sativus* (L.) belongs to Cruciferae family and is one of the most popular root crops that can successfully be grown throughout the year in the hills of Nepal. Being native to South-East Asia, the popularity of radish among the large number of farmers is due to its rapidly growing habit, short growing period, ease in cultivation and wider climatic adaptation. It is mainly cultivated for its fleshy root and tender leaves which can be eaten raw as salad or cooked as a vegetable (Shrestha, 2018). In Nepal, the cultivated area, production, and productivity of radish is 16915.7 ha, 2,68,119.6 tons and 15.9 t/ha respectively (Poudel, 2018).

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The growth and yield of radish greatly rely on soil and climatic conditions. Different varieties have different soil and climatic requirements for their optimum performance. Nepal having diverse agroclimatic conditions like temperate, subtropical and tropical (Malla et al., 2022), a single variety may not be suitable for all agroclimatic conditions (Degenhardt, 1982). Hence, identification of ecological niches of each variety is inevitable (Paudel, 2018). Farmers are growing very few recommended varieties of radish; however, many potential local radish cultivars are available in Nepal. Mino early, White neck, 40 days and Puthyane Red are released varieties of radish in Nepal (Chapagain, 2010).

Shrinking in its quality and yield are the results of diverse factors. The most quotidian and destructive disease of Brassicacea crops worldwide are Alternaria spp. such as Alternaria brassicicola (Schwein.) Wiltshire, A. brassicae (Berk.) Sacc., A. raphani J. W. Groves & Skolko, and A. alternata (Fr.) Keissl, mainly seed borne pathogens (Gautam, 2018). A. brassicicola and A. brassicae infect broccoli, brussels sprouts, cabbage cauliflower, Chinese, cabbage, kholrabi, kale, rutabage and turnip. A.raphani is the most often found on radish but can infect other brassica crops. Malla (2021) had put the disease and pest problem on 5th rank, as critical problem, arising in vegetable production, mainly radish. The most common symptoms of Alternaria disease is yellow, dark brown to black circular leaf spots a shot hole appearance. Individual spots coalesce into large necrotic areas and leaf drop can occur on petioles, stems, flowers, flower pedicels and seed pods. At least 20% of agricultural spoilage is caused by Alternaria species; most severe losses may reach up to 80% of yield. Among the root crops like radish, turnip, beet root and carrot, maximum infection (10-16%) has been found in radish and the disease has been widely spread in all the growing areas of Nepal. As the pathogen is plant parasitic in nature, it has become major problem in production of seed affecting the pod formation stage of crop and reducing the seed quality and size. The radish seeds infected with *A. raphani* may result in poor germination, a pre or post emergence blight, and evinces distinctive lesion in cotyledons and hypocotyles. There is presence of scab like lesions on table radish, and in the spotting and blighting of leaves, stalks and siliques. The most rapid progress of the disease occurred at temperatures within the optimum range for the fungus: 22-26°C. Experimental evidences suggest that *A. raphani* does not establish an overwintering inoculum in the soil by means of diseased plant debris. The lowest infection of *A. raphani* was found at 18°C with high moisture content in the soil (Atkinson, 1950).

Materials and Methods

Experimental sites

The experiment was carried out in the field of Campus of Live Sciences under Pathology Department in Tulsipur, Dang. It lies within the tropical agroecological belt, between latitude 28.1545°N and longitude 82.3235°E. The altitude of this ranges from 725m above the main sea level. The average temperature, precipitation, and relative humidity was 35°C/27°C (day/night), 23.64mm/day and 72% respectively (NASA-Power, 2020).

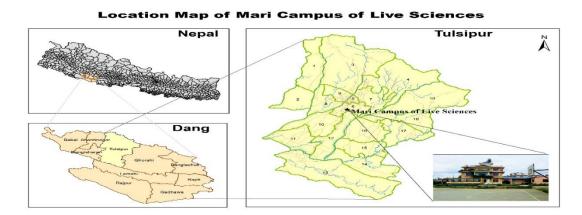


Figure 1. Research site of varietal screening of radish located at Mari Campus of Live Science, Tulsipur, Dang Source: Arc GIS

Table 1. Radish genotypes and their specifications

S. N.	Genotypes	Specification
1	Sparkle white F1 hybrid	Wider adaptability
2	Korean cross	-
3	Palak-patta 35	Excellent quality, edible leaves, marketable produce 30-35 DAS
4	Pyuthane red	Radish skin in color, Marketable produce 70-80 DAS
5	40 days	Marketable produce 35-40 DAS
6	Geeta-33	F1 hybrid
7	Mino early	Marketable produce 45-50 DAS

Experimental methods

The experiment was conducted in randomized complete block design with 7 genotypes of radish as treatments and each treatment were replicated thrice. There were altogether 21 plots with each plot size of 0.68 m² ($1.25m \times 0.55m$). Row to row and plant to plant spacing 25 cm and 10 cm respectively and the total area of experimental site was 42.88 m².

Experimental materials

7 genotypes of radish seeds were the materials used during the study. Seeds originating from different sources were obtained from authorized seed company. Experimental materials with their specifications are shown in the Table 1.

Field operations

Land preparation and Fertilization

One deep ploughing followed by 2 to 3 cross harrowing was done for the pulverization of the soil which was then levelled with the help of spade, hoe and rake before sowing. FYM was applied at the rate of 20 ton/ha (85.76 kg) as well as NPK at the rate of 80:60:80 kg/ha (2.24 kg N, 1.3 kg DAP, and 1.7 kg MOP respectively). Nitrogen was applied in three split doses (50% before sowing, 25% at 3 weeks after emergence, and last 25% at 5 weeks after emergence) while Phosphorus and Potassium was applied a day prior to sowing.

Seed sowing

Seeds were sown on 14th September at the rate of 10 kg/ha. Two seeds per hill at the depth of 1.5 to 3 cm were sown. There were 4 rows and 4 columns per plot with a total of 16 hills per plot.

Intercultural operations

During the entire study period, manual weeding was executed 2 times. Because of adequate rainfall during the study period, only light irrigation was provided at 10 days interval.

Disease Assessment

Disease Scoring

Incidence of disease was recorded after the appearance of disease while scoring was commenced after 10 days of disease appearance. And, three different scoring were executed at an interval of seven days using the scoring chart adopted by Townsend and Heuberger (1943).

Disease severity

From the score taken, disease severity was calculated per plot by following formula:

$$DS = \frac{SNR}{TPO} \times MR \times 100$$

Where,

DS : Disease severity SNR : Sum of all numerical ratings TPO : Total number of plants to observe MR : Maximum rating

Estimation of area under disease progress curve

The area under disease progress curve (AUDPC) was calculated by summarizing the progress of disease severity. The pattern of epidemic in terms of number of lesions, amount of diseased tissue, or number of diseased plants is given by a curve called disease progress curve, that shows epidemic over time and the area covered by this curve is known as AUDPC. AUDPC were computed, from the disease severities values from the formula given by (Shaner and Finney 1977), (Das et al., 1992).

AUDPC=
$$\sum_{i=1}^{N-1} [{Yi + 1} + Yi]/2][Ti+1 - Ti]$$

Where,

Yi= disease severity on first date Ti= date on which the disease was scored N= Number of dates on which disease was scored.

Data analysis techniques

Statistical analysis was performed with the M-Stat and MS-Excel. One-way ANOVA with DMRT (Dune'an's Multiple Range Test) was conducted to test for significant differences among genotypes for days to appearance of disease, incidence of disease, disease severity and reaction of genotypes based on average MDS and AUDPC.

Results and Discussion

Days to appearance and Incidence of Disease

Days to appearance at true leaf stage and incidence of Alternaria leaf spot disease in radish genotypes is shown in the Table 3.

From the perusal of the Table 3, it is evinced that significant difference among the genotypes for the appearance of disease was observed at true leaf stage while non-significant result was observed for the incidence of disease. It took maximum days for the Alternaria leaf spot to be visible in Mino early: 22 days after sowing (DAS) whereas disease appeared earliest in Pyuthane Red (18.33 DAS).

Our results for the days to appearance of disease contradict with the result of (Madhu Sudhan Ghimire, 2016) where they reported first disease appearance in the genotype 40 days on 24th days after sowing. This might be due the seasonal differences between these researched since our study was conducted during monsoon whereas their research was executed during the winter season. Our study revealed non-significant result for the incidence of disease among the different genotypes of radish. We obtained highest disease incidence on Pyuthane Red (34.8%) and this result is in conformity with the findings of (Madhu Sudhan Ghimire, 2016) where they reported Pyuthane Red as one of the varieties with highest incidence of disease.

Table 2. Disease score by Townsend and Heuberger (1943)

Scale	Infection	Host response
0	No symptoms	Highly resistance (HR)
1	Small light brown spot scattered covering $\leq 5\%$ leaf area	Resistance I
2	Spots small, brown, with concentric rings, covering 5% to 10% leaf area	Moderately resistant (MR)
3	Spots large, brown, irregular, with concentric rings, covering 10% to 25% leaf area	Moderately susceptible (MS)
4	Large, brown, irregular lesions with typical blight symptoms, covering 25% to 50% leaf area	Susceptible (S)
5	Large, brown, irregular lesions with typical blight symptoms, covering more than 50% leaf area	Highly susceptible (HS)

Table 3. Days to	disease appearance and	l incidence of <i>Alternaria</i>	<i>leaf</i> spot in radish genotypes
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Genotypes	Days to disease appearance	Disease incidence (%)
Sparkle white	19.67 ^{bc}	25.60
Korean cross	19.33 ^{cd}	29.80
Palakpatta-35	19.67 ^{bc}	29.10
Pyuthane red	18.33 ^d	34.80
40 days	19.67 ^{bc}	28.00
Geeta-33	20.67 ^b	28.10
Mino early	22.00 ^a	28.40
SEm (+/-)	0.34	
$LSD_{0.05}$	1.05	
CV	2.97	15.80
F test 0.05	*	NS

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1 able 4 Severity	I of Alternaria	leat snot disease	in radich	genotynes
Table 4. Severity	of michana	ical spot discuse	maansn	genotypes

Genotypes	Disease scoring (DS1)	Disease scoring (DS2)	Disease scoring (DS3)
Sparkle white	26.67°	35.00 ^b	44.17 ^b
Korean cross	34.17 ^{bc}	43.33 ^b	49.17 ^b
Palakpatta-35	25.83°	35.83 ^b	46.67 ^b
Pyuthane red	34.17 ^{bc}	47.50 ^b	55.00 ^b
40 days	57.33ª	64.67ª	70.33ª
Geeta-33	29.17 ^{bc}	43.33 ^b	54.17 ^b
Mino early	36.83 ^b	42.33 ^b	47.33 ^b
SEm (+/-)	2.98	3.86	3.39
LSD _{0.05}	9.19	11.90	10.35
CV	13.31	15.01	11.10
F test 0.05	**	**	**

Table 6. Mean Disease score and Mean AUDPC value of the radish genotypes

Genotypes	Mean Disease Score (MDS)	Mean AUDPC	Resistant category
Sparkle white	1.7 ^b	246.5°	Moderately resistant
Korean cross	2.1 ^b	297.5 ^{bc}	Moderately resistant
Palakpatta-35	1.8 ^b	252.3 ^{bc}	Moderately resistant
Pyuthane red	2.2 ^b	322.3 ^b	Moderately resistant
40 days	3.4ª	449.8 ^a	Moderately susceptible
Geeta-33	2.1 ^b	297.5 ^{bc}	Moderately resistant
Mino early	2.2 ^b	295.5 ^{bc}	Moderately resistant
SEm (+/-)	0.17	21	
$LSD_{0.05}$	0.53	64.71	
CV	13.31	11.78	
F test 0.05	**	**	

Disease severity

Severity of Alternaria leaf spot disease in genotypes of radish is shown in Table 4.

From the analysis of Table 4, it is revealed that significant difference was found among the genotypes found at each disease scoring: first, second and third scoring of disease.

During the first scoring of disease, the severity of disease was highest in 40 days (57.33%), whereas the lowest disease severity was found in Palakpatta-35 925.83%) which is statistically alike with the result obtained in Sparkle white (26.67%).

Similarly, during the second scoring, the disease was severe in 40 days (64.67%); on the other hand, the lowest severity was recorded in Sparkle white (35.00%) which is statistically similar with other remaining varieties.

Likewise, at final scoring of Alternaria leaf spot disease, maximum severity of disease was observed in 40 days (70.33%), while least severity was observed in Sparkle white (44.17%) which is statistically analogous with the result obtained in other cultivars.

Mean Disease Score and Mean AUDPC and reaction of genotypes

Mean score of the disease and the average area under disease progress curve (AUDPC) value of Alternaria leaf spot disease in radish genotypes is evinced in Table 5.

From the appraisal of the Table 5, it is unveiled that significant difference was found for both mean disease score (MDS) and mean AUDPC among the genotypes of radish. 40 days revealed significantly superior result for both the average score of Alternaria leaf spot disease (3.4) and mean AUDPC value (449.8) which indicate that it is moderately susceptible to Alternaria leaf spot disease; on the contrary, Sparkle White divulged significantly lowest average score of disease (1.7) and least average AUDPC value (246.5) heralding it to be moderately resistant among the genotypes of radish.

Our study divulged the highest average value of AUDPC in genotype 40 days with mean disease score of 3.4 which is in accordance with the findings of (Madhu Sudhan Ghimire, 2016) where they also recorded average AUDPC value on genotype Sparkle White (246.5) with 1.7 as mean score of disease. Similarly, 40 days was found to be moderately susceptible to Alternaria leaf spot while other varieties appeared to be moderately resistant, and no varieties were found to be completely resistant to the disease. Similar result was also reported by (Madhu Sudhan Ghimire, 2016; Subash Gautam, 2018) in their research where they found genotype 40 days and Pyuthane Red to be more susceptible than other genotypes.

Conclusion

From the current study findings, it can be demystified that severity of disease was found harsh in 40 days at each scoring, while severity was minor in Sparkle white. On assessing the mean scoring of disease and area under disease progress curve (AUDPC), 40 days was found to be moderately susceptible variety; however, other varieties were found to be moderately resistant to Alternaria leaf spot disease.

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