Nepal Agriculture Research Journal
Vol. 15, No.1, pp.106-114, 2023
DOI: https://doi.org/10.3126/narj.v15i1.51508

Integrated management of late blight of potato in Pokhara, Kaski, Nepal

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ARTICLE INFO

Research Paper

Received: September 01, 2022
Revised: November 16, 2022
Accepted: January 16, 2023
Published: February 01, 2023

Contents available at
http://www/sasnepal.org.np

Published by Society of Agricultural Scientists Nepal (SAS-Nepal).

ABSTRACT

A field experiments was carried out in Pokhara, Kaski, Nepal, to identify integrated disease management options against late blight of potato under natural epiphytotic conditions.Twelve genotypes of potato, including Janakdev as resistant and Cardinal as susceptible, were evaluated in a randomized complete block design with three replications. Similarly, a two-factor randomized complete block design experiment with three replications was carried out to investigate the interaction effect of planting date and application of chemical, botanical extracts, and a biocontrol agent.Susceptible variety Cardinal was planted in three different dates starting from October 11 at a 15-day interval and received five sprays each of the chemical fungicide Krilaxyl Gold (mancozeb 64% + metalaxyl 8%), 10% V/V extracts of garlic (*Allium sativum*), neem (*Azadirachta indica*), and bakaino (Melia azedarach), and Phytoderma (*Trichoderma viride*) 109 CFU/mL) and water spray as an untreated control.Among the genotypes, CIP384321.15 and Khumal Ujjwal showed the significantly ($p<0.01$) the lowest mean value of AUDPC (the area under the disease progress curve), whereas susceptible check Cardinal and Khumal Laxmi showed the significantly ($p<0.01$) the highest mean AUDPC values. Similarly, the 11 October planting with the application of Krilaxyl Gold showed the lowest total AUDPC value (382.65) and the highest total AUDPC value (1533.41). Besides the chemical fungicide, the application of extracts of garlic, bakaino, neem, and phytoderma could significantly reduce the disease and increase tuber yield. These botanical extracts and biocontrol agents can be used in the integrated disease management of potato late blight.

Keywords: AUDPC, Genotypes, date of planting, Integrated management, Late blight

Cited as: Adhikari M, S Shrestha, HK Manandhar and L Aryal. 2023. Integrated management of late blight of potato in Pokhara, Kaski, Nepal. Nepal Agriculture Research Journal **15**(1): 106-114. DOI: https://doi.org/10.3126/narj.v15i1.51508

INTRODUCTION

The potato (*Solanum tuberosum* L., 2n = 4x = 48) is one of the most important food crops for food security and ranks as the fourth staple crop in Nepal after rice, maize, and wheat. It is one of the major sources of income for smallholder farmers in the high mountain regions of Nepal (NPRP 2014; Timsina et al 2011; Khatri et al 2004) and a major vegetable and cash crop in the Terai and mid-hills. Different fungal, bacterial, viral, and nematode-incited diseases cause substantial yield loss in potatoes. Among them, late blight caused by *Phytophthora infestans* (Mont) de Bary is the most destructive disease affecting potatoes worldwide (Grooves and Ristaino 2000). It was first reported in Nepal between 1883 and 1897 and has been appearing as an epidemic since the mid-1990s (Ghimire et al 2003). A nationwide crop failure occurred due to late blight in 1996 (Dhital and Ghimire 1996). The estimated monetary loss due to this disease was reported to be approximately 104 million US dollars in 2009/2010, based on the total production of that year, an estimated 15% average loss, and an average potato price of 176 US dollars per tonne. Furthermore, a significant amount of resources are routinely spent in the Kathmandu valley to manage the crop by applying fungicides 10-15 times per crop during the autumn season (Sharma et al 2007).The disease poses a threat to food security, human health, and the environment in addition to causing economic losses (Kroman et al 2009). Under natural epiphytic conditions, screening of potato genotypes and determining the efficacy of different fungicidal agents in different planting dates were conducted to identify the integrated management option for late blight of potatoes.

MATERIALS AND METHODS

Genotypes screening experiment

The experiment was conducted in a farmer's field at Pokhara, Kaski, Nepal during November 2018 to February 2019. The site was situated at 28.2096° North and 83.9856° East longitude with elevation 1008 meters above sea level. Climate of the location was humid subtropical. Maize-crucifer cropping pattern was adopted in the field during the previous year. Sprouted tubers of 12 potato genotypes (LBR-14, Janakdev, MS42.3, IPY-8, CIP384321.15, CIP396311.1, PRP85861.8, BR63.65, Khumal Seto, Cardinal, Khumal Laxmi and Khumal Ujjwal (where, Cardinal as susceptible check and Janakdev as resistant check) obtained from Horticulture Research Station of Nepal Agricultural Research Council, Malepatan, Pokhara, were evaluated for their resistance to late blight disease in a randomized complete block design with three replications. Individual plot size was 1.5 m^2 and the area of the whole plot was 36 m^2. Each plot consisted of 2 rows and each row consisted of 5 plants.

Late blight disease management experiment

The experiment was conducted in a farmer's field at Pokhara, Kaski, Nepal during October 2018 to March 2019. Cardinal variety of potato, susceptible to late blight for the last few years, was used. Plants nutrients in the form of N, P_2O_5 and K_2O @ 100:100:60 kg ha^{-1}, respectively were applied through urea, di-ammonium phosphate and muriate of potash just prior to planting. Farm yard manure @ 20t ha^{-1} was also applied on the plot at the time of field preparation. Sprouted tubers of approximately similar physiological age were planted at 5-6 cm depth in ridges. Two flood irrigations at 30 and 45 days after planting were given. The study consisted of three different planting dates, starting from 11 October at 15 days interval and 5 sprays (each of fungicide, 3 different plant extracts and *Trichoderma*) and without spray as an untreated control in a two-factor randomized complete block design with 3 replications. Individual plot size was 4.2 m^2 and the area of the whole plot was 350.67 m^2.

There were 4 rows per plot at 60 cm apart and each row consisted of 7 plants. Plant to plant distance was 25 cm.

Factor A – Date of plantings
First date of planting (D_1): 11 October 2018
Second date of planting (D_2): 26 October 2018
Third date of planting (D_3): 10 November 2018

Factor B – Treatments
T1: Garlic (*Allium sativum*) extract 10% V/V
T2: Neem (*Azadirachta indica*) extract 10% V/V
T3: Phytoderma (*Trichoderma* sp.) 10^9 cfu mL^{-1}
T4: Krilaxyl Gold (mancozeb 64% + metalaxyl 8% (2 g L^{-1})
T5: Bakaino (*Melia azedarach*) extract 10 % V/V
T6:Untreated control (Water spray)

Preparation of botanical extracts
Fresh leaves of neem and bakaino, and cloves of garlic were soaked for about 5 minutes and grinded separately on a grinder. The crude fluid was filtered through a muslin cloth. The filtrate was collected and 100 mL extract each of neem, bakaino and garlic was separately diluted in 1000 mL water to make the final concentration of 10% V/V.

Application of treatments and scoring of disease
The treatments were applied by spraying after the first appearance of symptom of late blight in the field and continued for 3 times at 7 days interval. Ten plants from the middle of two rows were taken for assessment of the disease. Disease scoring was done using 0-5 scale (Sharma and Kolte 1994):

0 = No disease
1 = Less than 10% leaves of the plants infected with small lesions
2 = 10 – 25% of the plants infected with large lesions
3 = 26 – 50% leaves of the plants infected with large lesions and slight infection on the stem (less than 10%)
4 = 51 – 75% leaves of the plants infected with large lesions and more infection on the stem (11-50%)
5 = More than 75% of the plants leaf infected with large lesions, stem infection more than 50% plants, plants going to die

Disease severity

Ten plants were randomly selected to record disease severity from each experimental unit. Percentage disease severity was then calculated using the following formula (Cooke 1998).

$$\text{Disease severity (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Number of plants observed} \times \text{Maximum rating}} \times 100$$

Area under disease progress curve (AUDPC)

The area under disease progress curve was estimated using the following formula (Campbell 1990, Madden and Hughes 1995).

$$\text{AUDPC} = \sum_{i=1}^{n-1} (Y_{i+1} + Y_i) 0.5 (T_{i+1} - T_i)$$

Where,

Y_i = late blight disease severity % on the i^{th} date

T_i = date on which the disease was scored

n = numbers of dates on which disease was scored

Statistical analysis

Recorded data were tabulated using Microsoft excel program and processed using R studio software (version 1.1.463) for analysis, Microsoft excel program was used for data tabulation, and Duncan's multiple range test (DMRT) was carried out at 5% level of significance.

RESULTS

Screening of potato genotypes against late blight

The performance of screened potato against the late blight were highly significant ($p<0.01$) for AUDPC at 61, 68 and 75 days after planting (DAP) and mean AUDPC (Table 1). Significantly ($p<0.01$) high mean AUDPC values were found in Cardinal (485.54) and Khumal Laxmi (475.53) and significantly ($p<0.01$) the lowest AUDPC values were found in CIP384321.15 (105.88 and Khumal Ujjwal (143.47).

The test genotopes of potato also differed significantly ($p<0.01$) for tuber yield (Table 1). Highest yield was found in MS43.2 (17.42%) which was found statistically ($p<0.01$) at par with LBR 14 (15.96 t ha^{-1}), Janakdev (15.55 t ha^{-1}), IPY8 (14.42 t ha^{-1}), CIP384321.15 (14.33 t ha^{-1}) and Khumal Ujjwal (13.95 t ha^{-1}). Lowest yield was found in BR63.65 (10.75 t ha^{-1}).

Table1. Area under disease progress curve (AUDPC) values of late of potato under natural epiphytotic conditions and tuber yield at Pokhara, Kaski, Nepal, November 2018 to February 2019

Genotypes	AUDPC 1 (61 DAP)	AUDPC 2 (68 DAP)	AUDPC 3 (75 DAP)	Mean AUDPC	Yield (t ha^{-1})
LBR14	84.49^{ef}	173.76^{de}	265.40^{d}	174.55^{e}	15.96^{ab}
Janakdev	219.43^{b}	342.74^{bc}	434.08^{bc}	332.08^{bc}	15.55^{ab}
MS42.3	185.43^{bc}	262.83^{cd}	385.52^{c}	277.93^{cd}	17.42^{a}
IPY8	173.57^{bcd}	253.88^{d}	399.91^{c}	275.79^{cd}	14.42^{abc}
CIP384321.15	44.62^{f}	103.51^{e}	169.49^{e}	105.88^{f}	14.33^{abcd}
CIP396311.5	141.18^{cde}	248.79^{d}	389.99^{c}	259.99^{d}	11.73^{cd}
PRP85861.8	108.12^{def}	254.85^{d}	372.95^{c}	245.31^{d}	12.88^{bcd}
BR63.65	224.10^{b}	350.35^{b}	476.01^{b}	350.15^{b}	10.75^{d}
Khumal Seto	148.19^{cde}	234.33^{d}	382.07^{c}	254.31^{d}	13.68^{bcd}
Khumal Laxmi	359.53^{a}	469.85^{a}	597.20^{a}	475.53^{a}	11.60^{cd}
Cardinal	375.26^{a}	475.82^{a}	605.53^{a}	485.54^{a}	12.42^{bcd}
Khumal Ujjwal	65.08^{f}	131.56^{e}	233.77^{de}	143.47^{ef}	13.95^{abcd}
P value	5.38e-10	1.564e-08	2.169e-10	2.519e-10	0.007371
F test	**	**	**	**	**
LSD (0.05)	107.26	142.36	127	114.55	5.51
CV (%)	20.35	17.41	10.88	13.68	13.53
SEM(±)	17.77	20.21	21.96	19.68	0.41

CV= Coefficient of variation, LSD= Least significant difference, SEM= Standard error of mean difference, DAP: days after planting; Mean values followed by the same letter in the superscript are not significantly different according to the Duncan's multiple range test ($p<0.05$), **= Significant at $p<0.01$

Field management of late blight of potato

Effect of planting dates on total AUDPC and tuber yield

The effect of date of planting was highly significant (p<0.01) for AUDPC values (Table 2). Minimum total AUDPC value (756.65) was obtained for the first date of planting (11 October) and maximum total AUDPC value (1174) was obtained for the third date of planting (10 November). Similarly, the effect of date of planting was highly significant (p<0.01) for tuber yield (Table 2). Maximum tuber yield (10.65 t ha^{-1}) was obtained in early planted (11th October) plots while lowest tuber yield (7.57 t ha^{-1}) was obtained in late planted (10th November) plots.

Table 2. Effect of dates of planting on area under disease progress curve (AUDPC) of late blight and tuber yield under treatments application in Pokhara, Kaski, Nepal, October 2018 to February 2019

Date of planting	AUDPC 1 (61 DAP)	AUDPC 2 (68 DAP)	AUDPC 3 (75 DAP)	Total AUDPC	Tuber yield (t ha^{-1})
11 October	169.88^{c}	241.74^{c}	345.02^{c}	756.65^{c}	10.65^{a}
26 October	212.34^{b}	289.89^{b}	375.22^{b}	877.46^{b}	9.23^{b}
10 November	278.52^{a}	390.33^{a}	505.71^{a}	1174.56^{a}	7.57^{c}
Pvalue	0.0003147	0.000233	0.630676	0.0001415	0.00445
F test	**	**	*	**	***
LSD (0.05)	20.42	26.2	21.48	65.52	1.14
CV(%)	10	9.2	5.7	7.6	13.5
SEM (±)	31.61	43.77	49.3	124.16	0.89

CV= Coefficient of variation, LSD= Least significant difference, SEM= Standard error of mean difference, DAP: Days after planting; Mean values followed by the same letter in the superscript are not significantly different according to the Duncan's multiple range test (p<0.05), **= Significant at p<0.01, *=Significant at p<0.05

Effect of treatments on total AUDPC and tuber yield at different planting dates

The effect of treatments on both the total AUDPC values and tuber yield was highly significant (p<0.01) (Table 3). Garlic extract, neem extract, bakaino extract and *Trichoderma* ssubstantially reduced the disease and thereby increased the tuber yield compared with untreated control. However, the fungicide Krilaxyl Gold provided the highest protection from the disease with least AUDPC value (583.33) and thereby gave the significantly (p<0.05) highest tuber yield (12.29 t ha^{-1}).

Table 3. Effect of different treatments on total AUDPC values of late blight and tuber yield obtained for different dates of planting in Pokhara, Kaski, Nepal, October 2018 to February 2019

Treatments	AUDPC 1 (61 DAP)	AUDPC 2 (68 DAP)	AUDPC 3 (75 DAP)	Total AUDPC	Tuber yield (t ha^{-1})
Garlic extract 10% V/V	219.04^{c}	306.60^{c}	400.99^{c}	926.65^{c}	9.26^{b}
Neem extract 10% V/V	200.50^{d}	292.90^{c}	390.09^{cd}	883.49^{c}	9.07^{b}
Bakaino extract 10% V/V	174.94^{e}	265.09^{d}	368.52^{d}	808.56^{d}	9.59^{b}
Phytoderma 15 g L^{-1}	273.04^{b}	346.19^{b}	452.86^{b}	1072.10^{b}	7.81^{c}
Krilaxyl Gold	101.27^{f}	195.35^{e}	286.70^{e}	583.33^{e}	12.29^{a}
Untreated control	352.67^{a}	437.81^{a}	552.74^{a}	1343.22^{a}	6.86^{d}
P value	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2e-16
F test	**	**	**	**	**
LSD (=0.05)	16.34	21.29	23.46	45.22	0.56
CV(%)	7.7	7.2	6.0	5.0	6.4
SEM (±)	35.08	33.2	35.66	104.53	0.75

CV= Coefficient of variation, LSD= Least significant difference, SEM= Standard error of mean difference, DAP: Days after planting; Mean values followed by the same letter in the superscript are not significantly different according to the Duncan's multiple range test (p<0.05), **= Significant at p<0.01

Interaction effect of planting dates and treatments on total AUDPC values and tuber yield

Interaction effect of date of planting and treatment sprays on total AUDPC was highly significant (p<0.01) for tuber yield (Table 4). Significantly (p<0.05) maximum total AUDPC (1533.41) was obtained in untreated control plots planted on 10 November, while minimum total AUDPC value (382.65) was found in plots planted on 11 October coupled with spraying of Krilaxyl Gold. Similarly, minimum tuber yield (5.7 t ha^{-1}) was found in untreated control planted on 10 November and maximum tuber yield (14.66 t ha^{-1}) was obtained in plots planted on 11 October coupled with Krilaxyl Gold spray (Table 4).

Table 41. Interaction effect of planting dates and treatments on total AUDPC values of late blight and tuber yield of potato in Pokhara, Kaski, Nepal, October 2018 to February 2019

Treatments	AUDPC 1 (61 DAP)	AUDPC 2 (68 DAP)	AUDPC 3 (75 DAP)	Total AUDPC	Tuber yield (t ha^{-1})
11 Oct × Garlic extract	150.94^{fg}	220.08^{hi}	307.77^{h}	678.80^{h}	10.46^{cd}
11 Oct × Neem extract	140.94^{fg}	229.49^{hi}	352.16^{gh}	722.59^{h}	10.46^{cd}
11 Oct × Bakaino extract	131.11^{gh}	211.10^{hi}	335.25^{h}	677.46^{h}	10.86^{c}
11 Oct × Phytoderma	224.17^{e}	285.80^{def}	391.27^{efg}	901.25^{f}	9.11^{ef}
11 Oct × Krilaxyl Gold	65.83^{i}	112.37^{j}	204.44^{j}	382.65^{j}	14.66^{a}
11 Oct × Untreated control	306.27^{c}	391.60^{b}	479.23^{d}	1177.12^{c}	8.35^{fg}
26 Oct × Garlic extract	200.62^{e}	302.45^{cde}	405.09^{ef}	908.17^{ef}	9.27^{ef}
26 Oct× Neem extract	165.96^{f}	242.97^{gh}	333.72^{h}	742.65^{gh}	9.22^{ef}
26 Oct × Phytoderma	272.45^{d}	323.28^{cd}	387.97^{fg}	983.71^{de}	7.81^{g}
26 Oct × Krilaxyl	108.12^{h}	192.38^{i}	255.21^{i}	555.72^{i}	12.49^{b}
26 Oct × Bakaino extract	169.96^{f}	249.69^{fgh}	335.72^{h}	755.38^{gh}	10.03^{cde}
26 Oct × Untreated control	356.92^{b}	428.58^{b}	533.63^{c}	1319.14^{b}	6.52^{h}
10 Nov × Garlic extract	305.56^{c}	397.27^{b}	405.09^{ef}	1192.97^{c}	8.06^{g}
10 Nov × Neem extract	294.61^{cd}	406.23^{b}	484.38^{d}	1185.23^{c}	7.53^{g}
10 Nov × Phytoderma	322.50^{c}	429.48^{b}	579.34^{b}	1331.33^{b}	6.52^{h}
10 Nov × Krilaxyl	129.87^{gh}	281.28^{efg}	400.45^{ef}	811.61^{g}	9.71^{ef}
10 Nov × Bakaino extract	223.75^{e}	334.48^{c}	434.59^{e}	992.83^{d}	7.88^{g}
10 Nov × Untreated control	394.81^{a}	493.23^{a}	645.36^{a}	1533.41^{a}	5.70^{h}
p-value	0.00012**	0.01123*	0.00114**	0.00129**	0.03397*
LSD	28.31	36.88	40.64	78.33	0.97

CV= Coefficient of variation, LSD= Least significant difference, SEM= Standard rrror of mean difference, DAP: Days after planting; Mean values followed by the same letter in the superscript are not significantly different according to the Duncan's multiple range test (p<0.05), **= Significant at p<0.01, *=significant at p<0.05

Table 5. Resistant category of the potato genotypes based on the mean AUDPC values tested during November 2018 to February 2019 at Pokhara, Kaski, Nepal

Mean AUDPC Value	Level of resistance	Genotypes
0-100	Resistant	None
101-200	Moderately resistant	CIP384321.15, Khumal Ujjwal, LBR14
201-300	Moderately susceptible	Khumal Seto, PRP85861.8, CIP396311.5, MS, IPY8
301-400	Susceptible	Janakdev, BR
401-500	Highly susceptible	Cardinal, Khumal Laxmi

DISCUSSION

Of the 12 potato genotypes tested for their resistance to late blight under natural epiphytotic conditions, none was found resistant. Based on AUDPC values, the genotypes were categorized into moderately resistant to highly susceptible (Table 5). Only three and four genotypes showed moderately resistant and moderately susceptible reactions, respectively to the disease (Table 5). Sharma et al (2013) and Subedi et al (2021) also reported similar results for some of the genotypes, which were included in the present study. Also, it was observed (data not shown) that the disease had appeared late (53-56 days after planting) in these genotypes compared with the susceptible and highly susceptible genotypes (48-51 days after planting).Though the test genotypes significantly differed for tuber yield (Table 3), the differences are not considered for discussion because the levels of late blight differed from each other and the yield potential could also be varied for different genotypes. However, it showed that MS43.2, Janakdev and IPY8 had relatively higher tuber yield despite the AUDPC values were higher for these genotypes. It suggests that choice of varieties is important for disease management.

Decades of research on potato late blight have demonstrated that highly resistant (immune or nearly immune) phenotypes can frequently indicate an active major R gene (insert related reference/s), for which compatibility in the pathogen population is absent. If an incompatible potato genotype is released for use by farmers, there will be a selection of compatible pathogen population, which results in loss of resistance (Forbes 2012). For this reason, some researchers have recommended selection of those phenotypes which demonstrate resistance, (Forbes and Landeo 2006). So, the genotypes reacting as moderately resistant to moderately susceptible might provide durable and reasonable resistance to the disease, which could be effectively incorporated in integrated disease management practices.

The present study showed that the planting dates have significant relationship with late blight disease. Earlier plantings had significantly less disease (AUDPC values) than late planting. This could be due to low inoculum and unfavourable weather conditions for disease development in the earlier plantings. By the time the disease inoculum is enough and environmental conditions are favourable the earlier planted potato plants might escape the disease or they get less affected. Shrestha (1989) reported that the second or third week of October planting escaped the disease under Chitwan conditions. Gaire (2014) also reported minimum relative AUDPC value in potato planted in October planting and increased RAUDPC value in delayed planting.

Farmers do apply chemical fungicides regularly and too many times to protect potato crops from late blight diseases because these fungicides are effective in controlling the disease (Sharma et al 2011). Also in the present study, the fungicide Krilaxyl Gold found the best though other treatments such as extracts of garlic, neem, and bakaino and the biocontrol agent *Trichoderma* ssubstantially reduced the disease and increased the tuber yield compared with untreated control. Extracts of garlic and neem have been reported effective in reducing late blight severity both under vitro and in vivo conditions (Ngadze 2014, Mirza et al 2000). Also, extracts of bakaino has been reported for its antifungal activity against several phytopathogenic fungi such as *Rhizoctonia solani* and *Fusarium oxysporum* (Carpinella et al 2003). The present results demonstrate that these botanicals and *Trichoderma* can be used as components in integrated disease management of late blight under field conditions.

CONCLUSION

The genotype CIP384321.15 was found to be moderately resistant to late blight, with tuber yield on par with the MS variety, whose yield was found to be the highest. The resistance of Khumal Ujjwal was found to be on par with CIP 384321.15 and can be used as a source of resistance against late blight disease. Planting potatoes in October and foliar applications of Mancozeb (64% + Metalaxyl, 8%) were found to be effective in field management of late blight. However, given the residual toxicity of chemicals in the environment and human health, Bakaino extract, while less effective than Mancozeb (64% + 8%) and Metalaxyl (8%), which is comparable to neem extract and garlic extract, may be a better option for managing late blight disease in potatoes in an eco-friendly way for organic agriculture.

Acknowledgements

The authors express their gratitude to Horticulture Research Center for providing the potato germplasm. We also express our thanks to field staffs who kindly helped us during the field preparation for the experiment. Fund for this study was granted by DOREX, AFU.

Authors' Contributions

MA worked for selection of treatments, layout of design of experiment and carried out the experiment. MA collected field data and analyzed them while SS, HKM and LA provided regular guidance and supervision. MA made interpretation of the results and wrote the manuscript. All the authors approved the article before submitting to the journal.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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