Research Article

STUDY OF *Plasmodiophora brassicae's* VIRULENCE AND ITS MANAGEMENT ON CAULIFLOWER

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ABSTRACT

Club root disease is one of the most serious diseases affecting the production and the quality of cruciferous vegetables. The main aim of the study was to identify virulence of *Plasmodiophora brassicae's* pathotypes and assessment of efficacy of different treatments against clubroot disease. A field survey was conducted on 10 cauliflower's sample from Dhading, Makawanpur, Kavrepalanchowk, Lalitpur, Bhaktapur and Kathmandu, similarly laboratory experiment were conducted to assess the efficacy of different treatments. Disease inoculum was inoculated in 7 days old healthy seedlings of cauliflower and transplanted in the potting mixture with treatments applied. The virulence of each isolates and effectiveness of treatments along with differential hosts against clubroot was evaluated for disease incidence and severity. Isolates collected from Kavre (Panauti), Makawanpur (Palung) and Dhading (Benighat) showed highest disease incidence and severity. Similarly, Boron and Lime showed the least disease incidence and severity percentage, of 44.45% and 33.33% respectively followed by *Trichoderma* and Lime treatment with 48.14% and 37.03% disease incidence and severity respectively. Similarly, differential reaction among cole crops, cauliflower had highest incidence (100%) and severity (45%) followed by broccoli. While efficacy results of different treatments are derived from pot experiment so, it is needed to further validate by testing in disease infested fields.

***Keywords**: Clubroot, incidence, Plasmodiophora brassicae, severity, virulence*

INTRODUCTION

Clubroot is potentially the most serious disease of crucifer crops, especially cabbage/cauliflower and closely related crops. It is caused by a soil-borne phytopathogen *Plasmodiophora brassicae* Woronin (Wallenhammar, 1996). The organism is soil-borne and

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has long-lived resting spores that can survive in soil for more than 15 years. Clubroot has already made economic impact in many regions of the world (Dixon, 2009). The disease has been observed in Nepal since 1993 (Timila *et al*., 2008). The most of the crucifer vegetables are vulnerable to the infestation of clubroot disease in Nepal. The pathogen persists in soils for many years even if there is no suitable host and upon availability of susceptible crops, it builds rapidly. Increase in clubroot index is attributed in crop yield loss both in biomass and head/curd weight. The disease can cause yield loss of 27-81% in total biomass and 18-87% in curd yield of cauliflower, and can result in even 100 percent crop losses in severe cases (Timila, 2006).

The main objective of this study was to assess the virulence of *Plasmodiophora brassicae* isolates from different geographical regions and to test the efficacy of different treatments against clubroot.

MATERIALS AND METHODS

Research site: The research was carried out at the Central Agricultural Laboratory, Hariharbhawan, Lalitpur, Nepal.

Assessment of Clubroot disease severity collected from different Location

Commercial cauliflower fields from different location i.e. Dhading (Thakre, Benighat, Bisaltar and Malekhu area), Makawanpur (Palung, Tistung), Kavrepalanchowk (Panauti), Lalitpur (Khumaltar and Godawari), Bhaktapur (Nikosera) and Kathmandu (Kavresthali, Kirtipur, Phutung) were surveyed for clubroot and the roots of all plants within a 1 m^2 area were inspected at each of 10 locations along the arms of a W sampling pattern. The presence of galls was taken as an indication of *P. brassicae* infection, and clubroot severity was rated using a slightly improved grading standard (Peng *et al*., 2011) which included 0–3 scales: 0 = normal root growth without galling, 1 = galls on main roots or a few small galls formed on <1/3 lateral roots, 2 = galling on main root or on 1/3–2/3 lateral roots, and 3 = larger galls were formed on 2/3 of main root and lateral roots and used to calculate a disease index. Galled roots and soil samples were collected from each infested field, allowed to air dry, and stored in brown paper bags at room temperature until further processing.

The pH of soils collected from different region was determined in Soil and Fertilizer Testing Laboratory using pH meter and clubroot disease severity of the different samples from respective region was also determined and finally the correlation between pH and incidence of clubroot disease was also determined.

Virulence of *Plasmodiophora brassicae* pathotype collected from different diseased field

A total of 6 *P. brassicae* populations representing various geographical locations were selected for pathotype testing. Each population was derived from a collection of *P. brassicae* resting spores derived from a single plant from each field. Resting spores of *P.*

brassicae were extracted as described by (Tewari *et al*., 2005). Approximately 1 gm of dry root material was placed in a mortar and ground to a powder using a pestle. Twenty ml of sterile distilled water was added, and the homogenate was then mixed and filtered through six layers of cheese cloth. Concentration of resting spores was estimated with a haemocytometer and spore suspensions were diluted with sterile distilled water to 1×10^7 spores ml^{-1} for inoculation. One-week old seedlings of the Super White Top variety of cauliflower, which were pre-germinated on a piece of moistened filter paper in petri dishes, were inoculated by dipping the entire root system for 10 seconds in the resting spore suspension of six different isolates (Table 1) collected. The inoculated seedlings were then immediately planted in $6 \times 6 \times 6$ cm^3 plastic pots filled with manured soil at a rate of one seedling per pot, each treatment with three replication (each replication with three seedlings).

Three observations with two samples per replication were made for the disease score at 30^{th}, 45^{th} and 60^{th} day of inoculation in each treatment. Thus six samples per treatment were assessed at each observation.

Table 1. *Plasmodiophora brassicae* isolates based on location of sample collection

Treatments (Isolates)	Location
T1	Dhading-Benighat
T2	Makawanpur-Palung
T3	Kavrepalanchok-Panauti
T4	Kathmandu-Phutung
T5	Bhaktapur-Nikosera
T6	Lalitpur-Godawari

Efficacy of treatment against clubroot disease

In order to screen effective control measures gainst clubroot, application of different treatments selected based on their efficacy was done in the laboratory. Super white top variety of cauliflower seed was germinated in germination chamber. Seven days old seedlings were inoculated by dipping the entire root system for 10 seconds in the 1 × 107spores ml^{-1} resting spore suspension of *P. brassicae* as described earlier. Each pot was filled with the sterilized soil (sterilized by using 2% of formalin) and treated with different treatment as described below:

T1: *Trichoderma* and Lime

Indigenous isolate of *Trichoderma* extracted from soil collected from Panauti of Kavre District, was mass cultured in the laboratory using millet seeds and the *Trichoderma* grown on fingr millet seed was incorporated in sterilized vermicompost manure and kept at 28^0C temperature and moisture were maintained at 60% for effective growth of *Trichoderma*.

After 2 to 3 days a distinct whitish mycelial growth of *Trichoderma* was observed in vermicompost manure.

And for treatment purpose the potting was done by adding Lime as per recommended dose (i.e. 330 gm lime per ton of soil) and *Trichoderma* was grown in finger millet and applied in vermicompost @ 2 kg per 100 kg vermicompost.

T2: Lime
The addition of 330 gm lime (Calcium carbonate having 60% ECCE) in the sterilized 100 kg soil increased the soil pH from 6.2 to 7.3, measured for 10 days and that soil was used as the potting content.

T3: Boron and Lime
Amrit borax was used in the recommended dose 1Kg borax for 1 to 1.5 Ropani. For a single pot, almost 2 kg of soil was determined and the effective powdered form of boron was added in the lime applied soil for potting content. Amount of 0.10 gm of boron was used per pot along with the lime applied soil as described above.

T4: Cabbage Manure
Well decomposed cabbage manure was used as the treatment supposing the Isothiocyanates and the different other chemical content would suppress the clubroot of crucifers. Large amount of cabbage leaves was collected and was well decomposed by adding effective microorganisms. The well decomposed manure was incorporated in the sterilized soil in the ration of 1:1 and was used as the potting content for this treatment.

T5: Nebijin
Nebijin (Flusulfamide 0.3% DP) was used as the chemical fungicide. It was also applied in the potting mixture at the recommended dose of 300 kg/ha. The recommended amount of Nebijin was incorporated in the soil per pot. About 1gm of Nebijin was applied per potting content, estimated as per recommendation.

T6: Nebijin and Lime
As mentioned in the nebijin application treatment, Nebijin @ 1 gm per pot was incorporated in pot containing lime inoculated soil, as mentioned in the treatment.

T7: *Trichoderma*
The *Trichoderma* grown in the vermicompost manure as mentioned in the above treatment was incorporated in the sterilized soil @ 2 kg per 100 kg of soil and prepared for potting mixture.

T8: Control
Control treatment was prepared by using only soil (pH 6.2) with 20 ml water as the negative control.

Layout of the experimental design was RCBD with eight treatments and three replications. The research was conducted in laboratory conditions and the seedlings were transplanted in

treated pots. The treated pots with inoculated seedling were kept in controlled environment for 4 weeks. Three observations with two samples per replication were done for the disease score at 30th, 45th and 60th day after transplanting in each treatment. Thus six samples per treatment were assessed at each observation.

Differential reaction of host against clubroot disease

For differential host reaction study against clubroot disease, 6 plants of the cole crop family were taken as six different treatments and replicated three times (Table 2). The different host plants were germinated in the tray having cocopeat and vermicompost manure in 2:1 ratio. The germinated seedlings after 28 days were transplanted to the potting mixture after dipping their roots in resting spore suspension (1×10^7spores ml^{-1}) of *P. brassicae*.

Table 2. List of cole crops (treatment) used for differential reaction

Treatment	Common name	Scientific Name	Variety
T1	Mustard	*Brassica rapa*	Local
T2	Broccoli	*Brassica oleracea* var. *italica*	GBR-02
T3	Pak-Choi	*Brassica rapa* subsp. *chinensis*	Green wave
T4	Lettuce	*Lactuca sativa*	Hybrid
T5	Cress	*Lepidium sativum*	Hybrid
T6	Cauliflower	*Brassica oleracea* var. *botrytis*	Super White Top

Disease observation and statistical analysis

Three observations with two samples per replication were done for the disease score at 30th, 45th and 60th day after transplantation in each treatment. Thus six samples per treatment were assessed at each observation. Disease severity index (DSI) was calculated following Lahlali *et al.* (2013) and based on the grade standard. The data was analyzed using R software and Microsoft Excel.

RESULTS AND DISCUSSION

Assessment of clubroot disease severity collected from different Location

All fields had acidic soil to neutral soil, with mean pH values ranging from 5.6 to 7.1. The disease occurred in all cole crops producing districts under study but varied in severity (Fig. 1). The clubroot severity showed indirect relation with soil pH (r = -0.347). These results indicate that adjusting soil pH through liming is likely to delay infection and symptom development and upon integrating with other control techniques, can inhibit the formation of the root clubs (Fig. 2).

Application of cations to raise soil pH above 7.2 under greenhouse conditions reduced root hair infection and subsequent symptom development (Myers and Campbell, 1985; Webster

and Dixon, 1991). Raising the soil pH to 7.2 or above is a standard recommendation for clubroot management in vegetable crops (Calhoun, 1953). These reports support the finding of the study.

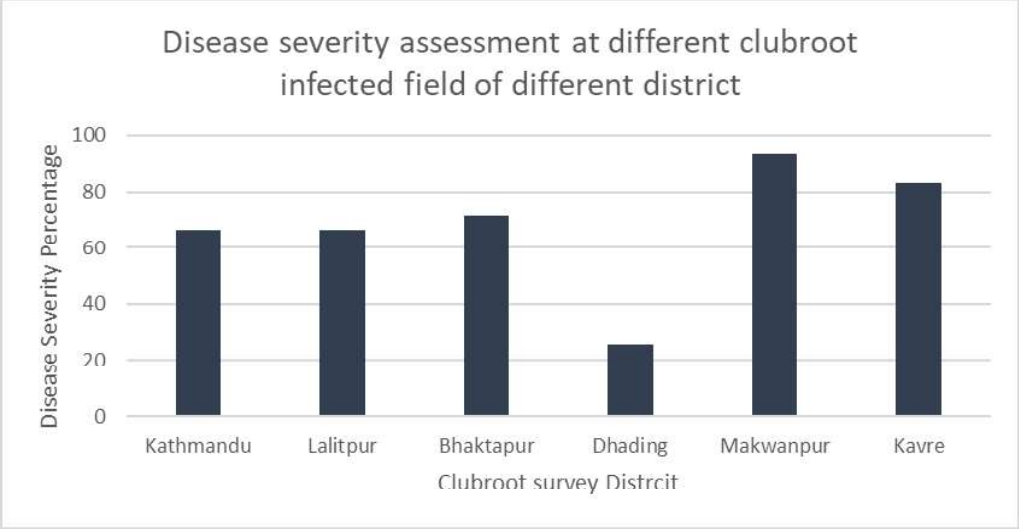

Fig. 1. Disease severity assessment of clubroot disease of different districts

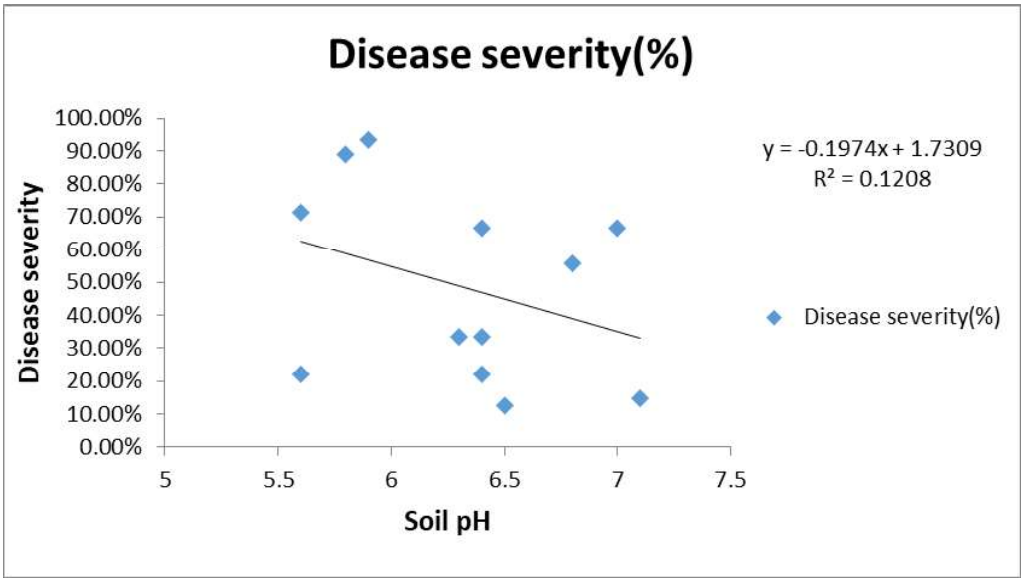

Fig. 2. Correlation of disease severity and soil pH

Virulence study of *P. brassicae* pathotype, collected from different diseased field

From the study of inoculated samples of cauliflower with different inoculum collected from different locations, it was found that disease incidence and disease severity percentage were found to be significantly influenced by different pathotypes collected. The highest disease incidence (100%) was recorded in seedling inoculated with inoculum collected from Kavre, Dhading and Makwanpur district at 65 DAI, however seedling inoculated with Lalitpur was found to have least disease incidence (50%) as shown in Table 3.

Table 3. Disease incidence percentage of inoculated seedling with different isolate collected at Hariharbhawan, Lalitpur, Nepal, 2021

Treatments	Disease incidence (%)		
	35 DAI	50 DAI	65 DAI
Dhading-Rorang	50^{b}	83.33^{a}	100^{a}
Makawanpur-Palung	50^{b}	100^{a}	100^{a}
Kavre-Panauti	100^{a}	100^{a}	100^{a}
Kathmandu-Phutung	50^{b}	83.33^{a}	83.33^{a}
Bhaktapur-Nikosera	50^{b}	100^{a}	100^{a}
Lalitpur-Godawari	16.67^{c}	50^{b}	50^{b}
SEM(±)	6.18	5.28	4.9
F-Probability	***	*	**
LSD	20.96	29.65	20.96
C.V.%	22.33	19.35	13.26
Grand mean	52.78	86.11	88.89

SEM± = Standard Error of Mean, CV= Coefficient of Variation, LSD= Least Significant Difference. Means in the column with the same letter (s) in superscript indicate no significant difference between treatments at 0.05 level of significance, '***' Significant at 0.001 level of Significance, '**' Significant at 0.01 level of Significance, '*' Significant at 0.05 level of Significance.

Similarly, it was found that disease severity of seedling inoculated with isolates from Makwanpur and Kavre district was highest, with 61.11 and 72.22 respectively at 65 DAI showing significant difference with other treatments. However, isolates from Lalitpur showed least disease severity at 35, 50 and 65 DAI (Table 4).

Table 4. Disease severity percentage of inoculated seedling with different isolate collected at Hariharbhawan, Lalitpur, Nepal, 2021

Treatments	Disease Severity (%)		
	35 DAI	50 DAI	65 DAI
Dhading-Rorang	16.67^{b}	27.78^{cd}	44.44^{b}
Makwanpur-Palung	16.67^{b}	44.44^{ab}	61.11^{a}
Kavre-Panauti	33.33^{a}	50^{a}	72.22^{a}
Kathmandu-Phutung	16.67^{b}	27.78^{cd}	27.78^{cd}
Bhaktapur-Nikosera	16.67^{b}	33.33^{bc}	33.33^{bc}
Lalitpur-Khumaltar	5.56^{c}	16.67^{d}	16.67^{d}
SEM(±)	2.06	2.93	4.76
F-Probability	***	***	***
LSD	6.18	12.10	13.97
C.V.%	22.33	20.41	18.44
Grand mean	17.59	33.33	42.59

SEM± = Standard Error of Mean, CV= Coefficient of Variation, LSD= Least Significant Difference. Means in the column with the same letter (s) in superscript indicate no significant difference between treatments at 0.05 level of significance, '***' Significant at 0.001level of Significance, '**' Significant at 0.01 level of Significance, '*' Significant at 0.05 level of Significance.

The high Disease Severity Index (DSI) of Makawanpur-Palung is supported by the research of Adhikari *et al*. (2020) showing the highest DSI in the control treatment (70.83%). Likewise, loss of a hundred million rupees in worth to clubroot disease within recent past few years was reported by Acharya and Gautam (2008) at Palung valley. Adhikari *et al*. (2020) also reported an outbreak of clubroot through the movement of seedlings in Palung of Makwanpur. The Plasmodiophoraceae family consists of 10 genera and 35 species (Braselton, 1995). Heterogeneity for differential pathogenicity of *P. brassicae* has been found in clubs from same infested field or within a club of same plant, showing that more than one pathotype may be present in a field (Jones *et al*., 1982). The result is also in line with these findings.

Efficacy of treatment against clubroot disease

It was found that some of the treatments were significantly different in disease incidence and disease severity. The highest disease incidence (80.23%) and disease severity (73.92%) was recorded in control treatment followed by Lime treated plants (77.78% disease incidence and 55.55% severity). The least disease incidence and disease severity was found on the boron and lime treated plants, with 44.45% and 33.33% respectively, followed by *Trichoderma* and Lime treated plant with 48.14% and 37.03% respectively. However, lime alone treated plant was found less effective. Similarly, sole application of Nebijn and

Nebijin in addition with lime were not found significantly different in one season cropping (Table 5).

Report from Hort-innovation (2018) had shown that the use of Boron had inhibited the infection and development of clubroot and Boron could be applied to the soil in formulation with Calcium nitrate fertilizer, which supports the finding of the study. Study by Cuevas (2011) also showed that treatment involving *Trichoderma* and Lime was found to be in least disease severity range in proportion with Boron and Lime. Nebijin, and Nebijin & Lime had controlled the disease to promising levels and the disease severity was also recorded to lower levels comparing the control treatments and similar results was earlier explained by (Timila, 2006). Cabbage manure also showed decrease in the severity of disease and controlled the disease at average levels as studied by Davies and Jones (2002) who observed the least incidence of the disease in the plots treated with Nebijin with respect to the control plots. The synthetic fungicides are attractive for Clubroot control and mercury-based fungicides are most effective although these have environmental toxicity (Peng *et al*., 2014).

Table 5. Disease incidence and disease severity percentage of inoculated seedling with different treatments at Hariharbhawan, Lalitpur, Nepal, 2021

Treatments	Disease incidence (%)	Disease Severity (%)
Trichoderma and Lime	48.14^{cd}	37.03c
Lime	77.78^{ab}	55.55b
Boron and Lime	44.45^{d}	33.33c
Cabbage Manure	51.85^{cd}	40.73bc
Nebijin	59.21^{cd}	48.14bc
Nebijin and Lime	55.54^{cd}	40.73bc
Trichoderma	62.96^{bc}	44.44bc
Control	80.23^{a}	73.92a
SEM (±)	2.98	3.05
F-Probablity	**	***
LSD	15.92	17.51
C.V.%	15.32	21.65
GRAND MEAN	60.02	46.77

SEM± = Standard Error of Mean, CV = Coefficient of Variation, LSD = Least Significant Difference. Means in the column with the same letter (s) in superscript indicate no significant difference between treatments at 0.05 level of significance, '***' Significant at 0.001 level of Significance, '**' Significant at 0.01 level of Significance, '*' Significant at 0.05 level of Significance.

Differential reaction of host against clubroot disease

It is seen in the figure that incidence and severity of clubroot was found almost negligible in Cress, however Pakchoi showed incidence of clubroot in the sample studied with disease severity of almost 80%. The Fig. 3 shows that among cole crops cauliflower had highest incidence (100%) and disease severity (45%) followed by broccoli.

In the present study, we investigated for the first time the combined effects of host resistance on clubroot resistance, and thereby potential effects on its durability. Diverse physiologic races based on the pathogenicity of *P. brassicae* isolates have been reported attacking crucifers (Ayers, 1957; Buczacki *et al*., 1975; Williams, 1966). Kuginuki *et al*. (1999) also observed intermediate scores on several differentials, and suggested that indistinct reactions were due to genetic heterogeneity of the hosts rather than the pathogen. *B. rapa* hosts were found to be infected only rarely by the clubroot pathogen (Toxopeus *et al*., 1986). On the *B. oleracea* hosts, all pathogen populations produced at least some disease, a finding also consistent with reports by Toxopeus (1986) and Voorips (1995).

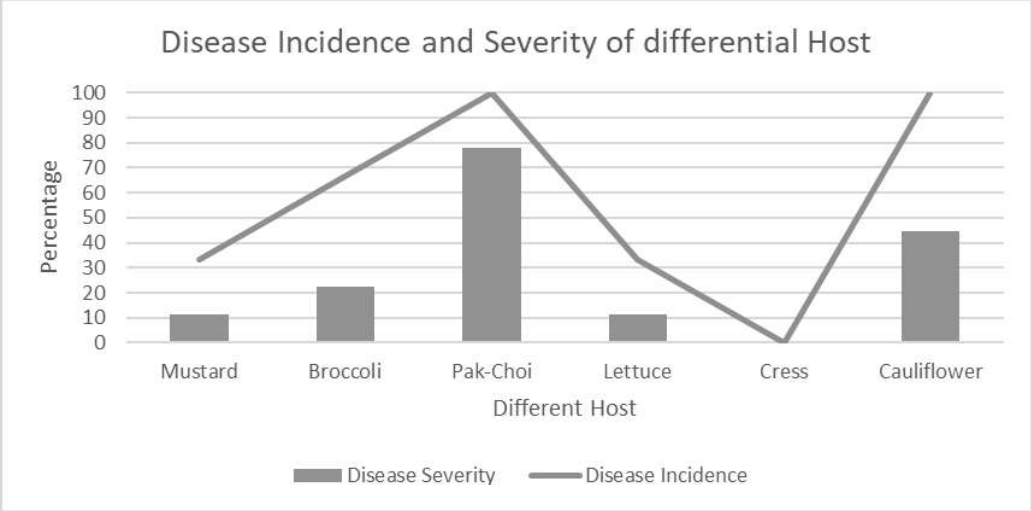

Fig. 3. Graph showing the disease incidence and disease severity of inoculated hosts

CONCLUSION

It was found that the highest disease severity was reported by inoculum from Kavre and Makwanpur followed by Dhading and inoculum from Lalitpur showed lowest. Similarly, soil pH was reported to have significant negative relation with the disease severity of *P. brassicae*. Likewise, the lowest disease incidence and severity was recorded on Boron and Lime and *Trichoderma* and Lime mixed treatments as compared to control. Furthermore, Nebijin mixed with Lime, and Cabbage manure also showed promising results. However, application of Lime alone was found ineffective in controlling the

clubroot disease as compared to other treatments. It was clearly seen from the study that chances of clubroot infestation in cress is rare, however disease incidence and severity was found highest in Pakchoi and Cauliflower crop as compared to other treatments. While the results of efficacy of different treatments are derived from pot trials, field trials in sick plot are essential to validate the findings of the study.

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