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Evaluation of Entomopathogenic Fungi against Potato Tuber Moth (*Phthorimaea operculella* ZELLER.) in West Shoa, Ethiopia

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Abstract

Potato Tuber Moth, *Phthorimaea operculella* is a major insect pest of potato (*Solanum tuberosum)* which has been identified to cause damage both under field and seed storage in Ethiopia. It is a major threat to potato production. A field experiment was carried out at Guder, Toke Kutaye districts to evaluate the effectiveness of different Entomopathogenic fungi and their concentration for the management of potato tuber moth (PTM). Treatment consisted of three species; *Beauveria bassiana* (PPRC-56), *Metarrhizium anisopliae* (MM), and *Verticilium lecani* at three level of concentrations ($1x10^6$, $1x10^7$, and $1x10^8$ spore/ml) with Diazinon 60% EC as a standard check and an untreated control. The treatments were laid out in a simple randomize complete block design (RCBD) with three replications. Results showed that *B. bassiana* significantly reduced the infestation of PTM larvae both on the leaves and tubers followed by the *M. anisopliae* when compared to *V. lecani*. Increasing the concentration levels of fungus also showed a significant reduction in the number of larvae and the damage caused on potato leaves. Based on the study findings, *B. bassiana* and *M. anisopliae* could be considered for the effective management of PTM in potato in the study area using higher fungal concentrations..

Keywords: Entomopathogenic fungi, Potato tuber moth, Concentration.

Introduction

Among the major insect pests reported in Ethiopia by Bayeh and Tadesse (1992) Potato Tuber Moth, *Phthorimaea operculella*, (Lepidoptera: Gelechiidae) is the most economically important pest of potato and caused damage both under field and storage conditions (Herman *et al*., 2005). In a study conducted at Alemaya, Eastern Ethiopia potato growing areas in 2001, the study revealed that, over 42% of potato tubers were infested by Potato Tuber Moth (PTM) and on the average, 8.7% of the potato tubers were lost due to field infestation, which has also been identify as the primary source of infestation in storage and later transfer back to the field (CIP, 1988).

To reduce field damage and further infestation in storage, minimizing practices that increase exposure of tubers from one place to another, use of different management strategies that are economical, appropriate and suitable to the environment, as well as haven significantly less or no adverse effect on non target organisms are recommended (Sileshi and Teriessa, 2001).

PTM infestation and damage on potato had often been controlled with insecticides for the past years and has rapidly developed resistance to a wider variety of insecticides, hence, the effective control of foliage attack with current insecticides has not prevented occurrence of high level of tuber infestation (Clough *et al*., 2008). The development of biological control strategies for the pathogen to reduce the infestation level and spread of PTM, *P. operculella* has thus become desirable even as there are limited work done on the management of PTM.

Therefore, the objective of this study was to evaluate the effectiveness of different Entomopathogenic fungi and concentration levels to reduce PTM infestation and damage.

Materials and Methods

Study area

The experiment was conducted in 2011 under irrigated condition at Ambo University research farm located in Gudar a distance of 125 km from Addis Ababa and about 12 km from Ambo town to the West. The site has an altitude of 2396 m.a.s.l. with an average annual temperature and rainfall of 19.5^{0}C and 900 mm respectively (Agricultural Office, 2002).

Evaluation of Entomopathogenic Fungi

Experimental design and treatments

The field experiment was laid out in a simple RCBD design with three replications. Treatment consisted of three types of fungal species *Beauveria bassiana* (PPRC-56), *Metarrhizium anisopliae* (MM), and *Verticilium lecani* at three level of concentrations (1x10^6, 1x10^7, and 1x10^8 spore /ml) and Diazinon 60%EC as a standard check with an untreated control. *Beauveria bassiana* (PPRC-56) and *Metarrhizium anisopliae* (MM) were obtained from Ambo Plant Protection Research Center (PPRC) while *Verticilium lecani* was from Holeta Agricultural Research Center (HARC). The plot size was 3.75m x 2.4m accommodating five rows of potato in each plot with a spacing of 75cm between rows x 30 cm between plants. Each plot was separated from the other by 1m pathway and 1.5m between replications. Eight medium size and well sprouted potato Jalane variety was used as planting material.

A routine microbial protocol developed by Fogal et al., (1986) was followed to mass culture and determine the concentration of spores of the pathogens. A hand held sprayer was used to spray the aqueous solutions on to the potato plots at 80 days after planting and this was subsequently repeated at two weeks interval to reduce the infestation of PTM.

Data collection and procedure

Data collected includes; pre spray count of the number of damaged leaves per plant and the number of larvae present per plant were recorded by taking 20 plants per plot randomly every week. The post spray recordings on potato plant leaves (damage leaves and larvae present per plant) was done weekly and continued until the time of vines clearing was reached.

The infestations on tubers were made by dissecting 50 tubers per plot which have been selected randomly to check for the presence of PTM larvae and damage status, which was measured in terms of feeding galleries carved per tuber present.

Ten days after the application of EPFs, larvae was collected from each of the treated plots and reared in laboratory to observe the dead, abnormal or deformed PTMs due to fungal infection. Dead ones were cultured on growth media to see development of pathogen on the dead insects. The petri dishes were prepared with water, 70 % ethanol and 5% Sodium hypochlorite. The dead insects were dipped one after the other, first in water, and then surface sterilized by dipping in 70% ethanol for three seconds and in 5% sodium hypochlorite for two minutes. The insects were rinsed in sterile and distilled water to kill any fungus on the surface of the insect before transferring to a petri dish prepared media of Sabouraud Dextrose Agar (SDAY) with yeast extract (Julia and Lina, 2010). Each Petri dish contained insects from the three concentrations and the three fungal species isolate were kept separately. The fungi that subsequently grow on the insect were observed and examined for the specific characteristics of the fungal species and the insect that had died from fungal infection.

Data analysis

Collected data were subjected to the analysis of variance (ANOVA) with JMP IN (2000) version: 5.1 and SAS (2002) version 9.00 program software. The different parameters were checked for normality and required transformation was done using logarithmic methods. The difference among treatments was determined using the Tukey-kramer test at 5% probability level.

Results and Discussion

PTM Larvae in Potato Leaves

The number of larvae counted per plant were significantly affected by the interactions of counting weeks with the type of treatments ($F_{40, 108}$ = 2.9, P<.0001). Result also showed that the larvae present was affected by the main factor of counting weeks ($F_{4, 108}$ = 57.3, P<.0001) and type of treatments ($F_{10, 108}$= 20.3, P<.0001).

There was no significant difference observed for the number of larvae counted on potato plant leaves treated by EPFs types at their different level of concentration for the pre spray count, and do not significantly differed from the standard check (Diazinon 60%EC) and the untreated control ($F_{10, 20}$ = 3.06, P>0.56). The number of larvae per plants counted on leaves a week after the first spray with *V. lecani*; applied at 1x10^{6}spore/ml was significantly higher than the other treatments except at the lowest concentration of *M. anisopliae* and at *B. bassiana*. These differences were from the standard check and not from untreated control ($F_{10, 20}$ = 30.48, P<0.0001). The highest concentration of *B. bassiana* and *M. anisopliae* recorded lower number of larvae and did not significantly differed from each other, as well as from *V. lecani* at the highest concentration. *B. bassiana* at the highest concentration was significantly different from the highest concentration of *V. lecani*. The numbers of larvae after two week treatment with *V. lecani* at 1x10^{6}spore/ml concentration and with *M. anisopliae* at 1x10^{6}spore/ml were not significantly different, but differed from *B. bassiana* at 1x10^{6}spore/ml ($F_{10, 20}$ = 20.5, P<0.0001). EPFs of *V. lecani* at concentration 1x10^7, 1x10^8 and *M. anisopliae* at 1x10^7, 1x10^{8}spore/ml and Standard check were not significantly different from each other, but differed from *B. bassiana* at 1x10^7, 1x10^{8}spore/ml and untreated control. The *B. bassiana* at 1x10^7 and 1x10^{8}spore/ml recorded the lowest number of larvae when compared with the other treatments. In the count made after a week from the second spray in *V. lecani* at 1x10^6 and *M. anisopliae* at 1x10^{6}spore/ml the number of larvae were not significantly different from each other when compared to other treatments. Lower number of larvae were recorded in plots treated with *M. anisopliae* at 1x10^8 and *B. bassiana* at 1x10^7, 1x10^8 spore/ml when compared to standard check and untreated control ($F_{10, 20}$ = 39.7, P<0.0001). (Table 1).

The result of larvae present on potato leaves counted at time of spray by the type EPFs at different concentrations were compared and presented in Table 1 across in rows. The number of larvae counted for *B. bassiana* for the infestation of PTM pre spray at the concentrations 1x10^6 ($F_{4, 10}$ = 77.03, P<.0001), 1x10^7 ($F_{4, 10}$ = 36.9, P<.0001) and at 1x10^8 spore/ml ($F_{4, 10}$ = 51.3, P<.0001) to the last counts of post spray there were significantly reduce than the *V. lecani* at 1x10^6 ($F_{4, 10}$ = 25.1, P>0.008), 1x10^7 ($F_{4, 10}$ = 9.6, P>0.004) and at 1x10^8 spore/ml ($F_{4, 10}$ = 30.4, P<.0001) concentrations followed by *M. anisopliae* at 1x10^6 ($F_{4, 10}$ = 6.5, P>0.007), 1x10^7 ($F_{4, 10}$ = 12.9, P<.0001) and at 1x10^8 spore/ml ($F_{4, 10}$ = 60.2, P<.0001) concentration. In general, the spraying of the different concentrations of the EPFs when applied repeatedly, significantly reduced the level of PTM larvae infestation on leaves in treated plots.(Table 1). Lower concentration caused very low reduction in the number of PTM larvae when compared with the two higher concentrations which reduce foliar infestation significantly.

The result agreed with the findings of Hassani (2000) who reported that *B. bassiana* significantly reduced the population of *Helicoverpa armigera.* Al-Deghari in 2008 also reported that *B. bassiana* germinated rapidly, sporulated very well and showed high virulence and good discharge of conidia, which demonstrated the high efficiency of the EPF in controlling a target organism. Similarly, the results from the study of the three types of EPFs showed that *B. bassiana* gave the higher response against of PTM than *M. anisoplae* and *V. lecani* when tested under field condition.

Table 1. Effect of EPF sprayed at different time on larval number of PTM

EPFs	Conc. Spore/ml	Number of larvae counted per plant (Mean±SE)					F-ratio	P-value
		Pre spray	Post spray count					
			Post spray 1:1	post spray 1:2	post spray 2:1	post spray 2:2		
V. lecani	1x10[6]	1.4±0.18aA	1±0.03baBCDE	0.7±0bCDE	0.5±0.0bDE	0.3±0.02cbE	25.1	0.008
	1x10[7]	1.6±0.4aA	0.7±0cBCD	0.5±0dceCDE	0.4±0.0cdDE	0.2±0.02cdE	9.6	0.004
	1x10[8]	1.4±0.19aA	0.6±0dcBCD	0.5±0dcCDE	0.3±0.0eDE	0.1±0.03eE	30.4	<.0001
M. anisopliae	1x10[6]	1.4±0.35aA	0.92±0.03bBA	0.8±0.1bCAB	0.6±0.0bDABC	0.27±0.04cbEABCD	6.5	0.007
	1x10[7]	1.29±0.3aA	0.7±0.04dcBCD	0.5±0.1dceCD	0.3±0.0edDE	0.07±0.04eE	12.9	<.0001
	1x10[8]	1.28±0.12aA	0.58±0.02deBC	0.4±0.03deCD	0.2±0.1fDE	0.03±0.02eE	60.2	<.0001
B. bassiana	1x10[6]	1.4±0.0aA	0.9±0.03bB	0.6±0.03cCD	0.4±0.02edD	0.2±0.03edE	77.03	<.0001
	1x10[7]	1.3±0.2aA	0.6±0.01deBC	0.3±0.02eCDE	0.2±0.05fDE	0.1±0.02eE	36.9	<.0001
	1x10[8]	1.27±0.13aA	0.5±0.00eBC	0.3±0.04deCDE	0.1±0.01fDE	0.1±0.02eE	51.3	<.0001
Diazinon 60% EC		1.5±0.19aA	0.7±0.02eBCDE	0.5±0.02cCDE	0.5±0.04cbDE	0.4±0.04E	25.3	<.0001
Untreated		1.6±0.15aA	1±0.02aABC	1.3±0.03aBC	0.87±0.02aCD	0.75±0.03D	12.5	0.0007
F-ratio		3.06	30.48	20.5	37.9	27.2		
P-value		0.56	<.0001	<.0001	<.0001	<.0001		

* Means ± SE followed by the same letter (s) within column (lower case letter) and rows (upper case letters) are not significantly different from each other at P<0.05.

* **Post spray 1:1** = First spray after a week, **Post spray 1:2** = First spray after two week, **Post spray 2:1** = Second spray after a week, **Post spray 2:2** =Second Spray after two week.

PTM larvae in Tuber

The mean number of larvae counted was found to be significantly affected by the applied treatments of EPFs at the different level of concentrations ($F_{10, 20}$ = 20.1, P<.0001). Potato tubers from plots treated with *V. lecani* at 1x10^{6}spore/ml was significantly different from all other treatments applied and did not differed from the untreated control on number of larvae per tuber counted. *V. lecani* at the highest level of concentration did not differed from *M. anisopliae* at 1x10^{7} and *B. bassiana* at 1x10^{6}spore/ml. (Table 2).

Damaged tubers

Percent damaged of tubers counted show, very highly significantly differences were observed between the treatments applied $F_{10, 20}$ = 25.84, P<.0001). The percent damaged in tubers of plots treated ranges from 9.7% to 60.7%. The highest of tuber damage was recorded in *V. lecani* at its lowest concentration level; the next highest tuber damage was in *V. lecani* at 1x10^{7} and *M. anisopliae* at 1x10^{6}spore/ml (53%). The *V. lecani* at highest level was also not significantly differ from *B. bassiana* at the lowest level. *B. bassiana* at 1x10^{7} and 1x10^{8} were recorded the lowest tuber damage and not significantly differ from each other, but significantly differ from all treatments applied as well as from the standard check and untreated control (Table 2).

Carved galleries on tubers

The mean number of galleries was found significantly affected by the treatments of EPFs at their different level of ($F_{10, 20}$ = 14.38, P<.0001). In plots treated with *B. bassiana* at 1x10^{8}spore/ml was not significantly different from the *B. bassiana* at 1x10^{7}spore/ml, but significantly differed from the standard check as well as untreated control by recording the lowest number of galleries per tuber. *B. bassiana* at 1x10^{7} was also not different from *M. anisopliae* at 1x10^{8} and 1x10^{7}spore/ml. (Table 2).

The EPF foliar spray significantly reduced larval infestation on leaves, and further damaged in tubers during harvesting. The number of larvae present in tubers, percent of damaged recorded and carved galleries created on tuber of damaged by PTM larvae, in all the three concentrations for the three EPFs showed a significant reduction in tuber damage.

The damaged tubers by PTM showed that the potatoes treated with *V. lecani* were not well protected from PTM damage, but the tuber treated with *B. bassiana* and *M. anisopliae* were significant and effective in reducing tuber damage by PTM. When the concentrations were compared, high percentages of tubers were damaged at lower than at higher level. Number of galleries counted in tuber of PTM treated EPF on the field decreased as the level of concentration increased within each type of EPFs. *B. bassiana* significantly reduced the larval counted, percent of damaged tuber and number of carved galleries compared to *M. anisopliae* and *V. lecani* .The result agrees with the findings of Julia and Lina (2010) who reported that an increase in the concentration of *B. bassiana and M. anisopliae* spores from $1x10^5$ to $1x10^7$ increased the mortality of *Acanthoscelides Obtectus.* Moreover, Karthikeyan and Selvanarayanan (2011) through bioassays of *B. bassiana* against *H. armigera* reported that the percent mortality of the insect increased linearly with increase in doses of the EPFs.

Table 2. Effect of EPF at different level of concentration on PTM and damage on harvested tuber

EPFs	Conc.(Spore/ml)	PTM recorded (Mean±SE)		
		Larvae/ tuber	% damage tuber	Galleries/ tuber
V. lecani	$1x10^6$	0.8±0.03a	60.7±3.3a	1.5±0.1ba
	$1x10^7$	0.6±0.0b	53.5±4.1a	1.8±0.1a
	$1x10^8$	0.4±0.03cd	38±4.2b	1.1±0.1dc
M. anisopliae	$1x10^6$	0.6±0.03b	53±2.5a	1.6±0.12a
	$1x10^7$	0.4±0.03cd	30±5.8cb	0.95±0.2dce
	$1x10^8$	0.2±0.0ef	23.3±2.4cd	0.8±0.07dce
B. bassiana	$1x10^6$	0.4±0.03d	38.7±5.04b	1.2±0.08bc
	$1x10^7$	0.2±0.02ef	18.3±2.7ed	0.6±0.08fe
	$1x10^8$	0.1±0.02f	9.7±1.7e	0.3±0.04f
Diazinon 60% EC		0.31±0.05cd	24.67±10.97cd	0.65±0.25de
Untreated		0.72±0.06a	66.67±6.36a	1.83±0.12a
F-ratio		20.11	25.84	14.38
P-value		<.0001	<.0001	<.0001

* Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05.

Testing of fungal infection on PTM

The fungal type which was applied in plots by *B. bassiana* record highest percent of mortality of larvae (60% in 1st test, 68% in 2nd test and 80% in 3rd test) in all the three tested (reared) than the *M. anisopliae* (49% in 1st test, 57% in 2nd test and 76% in 3rd test) and *V. lecani* tested (44% in 1st test, 54% in 2nd test and 70% in 3rd test). The mortality of larvae increased from 1st test to the 3rd tests in all fungal type, where none mortality was recorded in control group (only 1% in 2nd test and 0% in 1st and in the 3rd mycosis test), meaning that the dead larvae in treatment with fungal type died by fungal infection (Figure 1).

During the mycosis test conducted in laboratory from the infested PTM larvae in the field by the EPFs applied showed that the mortality larvae of PTM larvae mortality increased from the first tested to the third test time in all the three type of EPFs. The higher mortality of PTM was recorded with *B. bassiana* type of EPFs than the *M. anisopliae* and *V. lecani.*

The dead PTM larvae had various morphological deformities and abnormalities ranging from blackening of the infected skin, formation of fragile skin to development of abnormal body parts. In addition, the larvae revealed characteristic of deformities related with improper cuticle formation or due to severely attacked cuticle. Butt (2001) and Arora, *et al.*, 2000) reported that when spores from *B. bassiana* come into contact with insect's cuticle, they caused a disease called *Muscardine*, because of the characteristic white mould. The conidium of *M. anisopliae* under favorable condition germinates into a short germ tube which gives out small swelling called appressorial, the insect infected by this fungus were killed mainly by histolytic action of fungus and the excessive mycelium growth which mechanically blocked the gut and exert physical pressure on it and other vital organs in general (Kannan *et al*., 2008; Zimmermann, 1993).

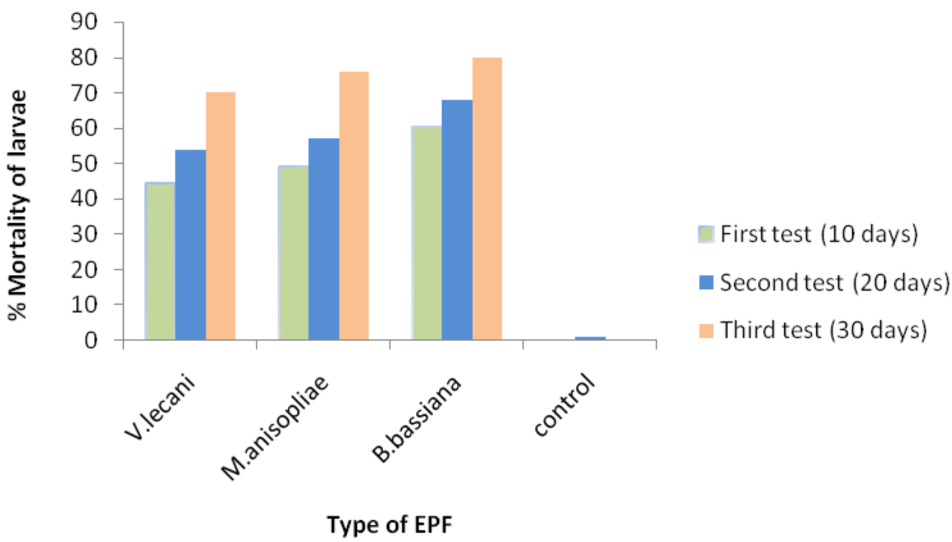

Figure 1: Mortality of PTM larvae with EPFs in field tested at 10 days interval in laboratory.

At the end of the fungal infection the different morphological abnormality of PTM dead larvae and the sporulation of fungus from the dead body were observed. Dead and shirked body skin, blacking of body, abnormal and deformed; on treated PTM larvae under present studies (figure 2**).**

Figure 2. Morphological abnormalities and sporulation EPFs on PTM larvae

Yield and yield related components

The yield and yield components result before and after harvesting tuber during the evaluation of EPFs under the field are presented in Table 3. The Tuber per plant counted at harvesting time were significantly affected by the EPFs at the different concentration level ($F_{10,\ 20}$ =30.25, P<.0001). The highest concentration recorded the highest number of tuber per plant for all types of EPFs. *B. bassiana* at the 1x10^8spore/ml recorded significantly higher number of tuber when compared with all other treatments. *M. anisopliae* at 1x10^6 was not significantly different from the untreated control by recording the minimum number of tuber per plant (Table 3).

Yield in tonne/ha was significantly affected by the applied treatments ($F_{10,\ 20}$ =5.82, P<.0001). The yields harvested from *V. lecani* at 1x10^8, *M. anisopliae* at 1x10^7,and 1x110^8 and *B. bassiana* at 1xs$10^7$and 1x10^8spore/ml treated plots were the highest and significantly differed from the standard check and the untreated control (Table 3).

Average weights of tuber in gram per plant were significantly affected by the EPFs applied at different level of concentrations ($F_{10,\ 20}$ =9.3, P<.0001). The highest average of weights were recorded in *B. bassiana* at 1x10^8 and *M.*

anisopliae at 1x10^{8}spore/ml and there were significantly differ from the other level of treatments and also from the standard check as well as from untreated control (Table 3) .

Table 3. Effects EPFs and their concentration levels on yield and yield related components

Treatment		Yield components (Mean±SE)		
		TPP (g)	YPH (t/ha)	AWT (g/plant)
	1x106	13±0.4e	22.7±0.2ed	79±5e
V. lecani	1x107	16±1de	29.6±0.4bedc	95±3cd
	1x108	19.4±1c	38.8±0.4ba	113±7bc
	1x106	16±0.3de	20±7.6e	89±2ed
M. anisopliae	1x107	19±0.4c	35.6±0.2bac	100±2bcd
	1x108	23.5±1b	40.3±0.3ba	117±3ba
	1x106	18.4±0.5c	30.3±0.9bedc	101.2±1bcd
B. bassiana	1x107	23.7±1b	35.2±0.4bac	111.8±3bc
	1x108	28.5±0.4a	45.4±1.6a	130.3±7a
Diazinon 60% EC		17.07dc	30.9bdc	100.2bcd
Untreated		13.3e	24.9edc	76.95e
F-ratio		30.25	5.82	9.3
P-value		<.0001	0.0004	<.0001

* Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05.

* **TPP**= Tuber/Plant, **YPH**=Yield/Hectare (tone), **AWT**=Average Weight of Tuber (gm),

Conclusions

The results obtained in this study revealed that the tested fungal species isolates were effective against potato tuber moth and resulted in causing high mortality and reduction in infestation. Increasing the concentration of spores/ml was found to generally decreased the number PTM larvae and their damage on leaves and tubers. There were also liner relationships between concentrations, number of larvae, damage to tubers and number of galleries carved by PTM larvae. *B. bassiana* (PPRC-56) isolate had the highest virulence to invade the PTM in the field followed by *M. anisopliae* (MM) and by *V. lecani.* The findings indicated that for effective control of *P. operculella* in potato, *B. bassiana* and *M. anisopliae* could be developed as biological control alternative to conventional synthetic insecticides in integrated pest management program. The use of higher fungal concentrations is advantageous for better management of PTM. Entomopathogenic fungus as biological control agents to control insect pests might have effect on beneficial insects, such as natural enemies of insect pests, these needs to be further investigated. The investigation of EPF isolates at different location in the potato growing highland areas of Ethiopia to now the ecological ranges of EPFs isolates for the management of PTM should be given more attention.

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