



Carboxylesterase activity of filamentous soil fungi from a potato plantation in Mankayan, Benguet

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Abstract

In this study, filamentous fungi were isolated from a soil sample from a farm in Mankayan, Benguet. The isolates were tested for the presence of carboxylesterase enzyme as it would indicate the ability to breakdown pyrethroid pesticides such as Cypermethrin. A total of fourteen fungal isolates were characterized morphologically and were identified using the D1/D2 regions of 28S rDNA. All were identified to be members of the Ascomycetes. Seven of the isolates belong to the genus *Fusarium*, and two were identified to be *Aspergillus heteromorphus* and *Penicillium* sp. All fourteen isolates exhibited carboxylesterase activity. Isolates BDP3 and BDP10 exhibited the greatest carboxylesterase activity. These two isolates, both unidentified Ascomycetes, are promising species for mycoremediation specifically targeting pyrethroid pesticides.

Key words – *Aspergillus heteromorphus* – carboxylesterase – cypermethrin – pyrethroids

Introduction

The potential for bioremediation by fungi, also known as mycoremediation, is largely ignored. Singh (2006) says in his review paper that there is shortage of reports involving fungi in bioremediation because the biology and ecology of fungal metabolic processes are rarely examined. There is no shortage, however, in references that describe fungi as ubiquitous and highly adaptive organisms. These characteristics of fungi make mycoremediation technology applicable even in harsh conditions. One possible field of application can be found in modern farming, where the regular use of chemicals as pesticides and fertilizers is the norm. Such is the case for vegetable farms in Mankayan, Benguet. In this province, at least 54% of the labor force is engaged in vegetable and cut flower production. It is the leading economic activity of the province (Province of Benguet Official Website 2014). Mankayan specifically devotes 7,515 hectares (58%) of its total land mass to agriculture, the key crops being highland vegetables (DILG-CAR 2014). Crops that are notably produced in Mankayan and other municipalities of the province include potatoes, carrots, broccoli, cauliflower, Chinese cabbage, and bell pepper. These vegetables are of temperate variety that are vulnerable to pest attacks such as borers and aphids. As such, farmers resort to pesticide use to drive away or kill these pests that decrease their yield and eventually their income (Piadozo 2008).

Pesticides may be classified according to their mechanism of action. They disrupt the nervous system (insecticides), target the photosynthetic pathway (herbicides), and other biosynthetic reactions (fungicide, bactericide) (DeLorenzo et al. 2001). The use of these pesticides does increase the yield for the farmers. However, researches have demonstrated that pesticides can also be toxic to other non-target organisms, both terrestrial (Mahmood et al. 2016) and aquatic (Antwi & Redd 2015). Many of the other organisms affected are beneficial, i.e. mycorrhiza affected by Benomyl fungicide (Channabasava et al. 2015). Deleterious effects of pesticides include reduced survival, growth and reproductive rate due to exposure to sub-lethal dosages (Ortiz-Hernandez et al. 2011).

Approximately 90% of pesticides do not even reach their target. Rather, they are dispersed in the air, soil and water (Ortiz-Hernandez et al. 2011) and thus, traces of it could be routinely detected especially in soil. Ecologically, pesticides disrupt the balance in the environment. To humans, adverse effects to health include poisoning, allergy, abdominal and chest pain, nausea, anorexia and fatigue and, in the most severe cases, fasciculation in large muscles of the extremities (World Health Organization 2005).

There are many synthetic compounds currently used as pesticides. They may be classified according to the target pest, the chemical structure of the compound used, or the degree or type of health hazard involved (Gevao et al. 2000). Among these include Gamma-hexachlorocyclohexane (γ -HCH) (Quintero et al. 2007), chlorpyrifos (Fang et al. 2008), and pyrethroids (Sakata et al. 1992). Synthetic pyrethroids are particularly interesting because it is a class of more than 1000 powerful, broad-spectrum insecticides used to control insect pests in agriculture, households, and stored products. (Mueller-Beilschmidt 1990). This insecticide is used worldwide in agriculture, forestry, horticulture, public health (i.e., hospitals, vector control), and residential pest control (e.g. landscape maintenance, structural pest control) (Werner & Young 2017). But while these kill pests, it also poses a threat to the environment. Effects similar to those in insects have been observed in other organisms such as frogs that have ingested the insecticide. It is highly toxic to fish, bees, and water insects (United States Environmental Protection Agency 1989). In addition, its high persistence in the environment due to its hydrophobicity, stability in sunlight, and low volatility (amplifies its detrimental effects to the environment (Werner & Young 2017). The half-life of cypermethrin in the soil can range from two to eight weeks (United States Environmental Protection Agency 1989).

Fungi are prime candidates for soil bioremediation as they are very ubiquitous in this habitat (Maqbool et al. 2016). Studies by Fang et al. (2008), Quintero et al. (2007), Silambarasan & Abraham (2013), attest to the suitability of fungi to reduce the rate of soil pesticide pollution by discovering the activity of soil fungi in lowering the concentration of hexachlorocyclohexane and chlorpyrifos. The basidiomycete *Trametes versicolor* was also found to have successfully degraded imiprothrin, oxytetracycline, cypermethrin, and carbofuran (Mir-Tutusaus et al. 2014). Even the marine fungi *Aspergillus sydowii* and *Penicillium decaturense* were found to degrade methyl parathion (Alvarenga et al. 2014).

In a similar way, pyrethroids (including cypermethrin) are also vulnerable to enzymes that catalyze its cleavage (Leahey 1985, Sakata et al. 1992). The enzyme with this specific function is carboxylesterase (CES). This enzyme efficiently hydrolyzes a large number of endogenous and xenobiotic ester-containing compounds like the pyrethroids (Wang et al. 2009). In the study of Wheelock et al. (2006), they discovered that CES applied to water spiked with permethrin and bifenthrin was able to remove toxicity to *Ceriodaphnia dubia* by 1,000 ng/L and 600 ng/L, respectively.

Fungi, together with the enzymes they produce (i.e carboxylesterase for pyrethroids), are very important in the bioremediation of environmental contaminants such as pesticides. They are the natural mode of decreasing the possible adverse effects of pesticide pollution. In this research, filamentous fungi exposed to cypermethrin (a pyrethroid) were identified and screened for the production of carboxylesterase. This will give us an idea of the level of resilience of environments chronically polluted with pesticides such as pyrethroids and the possible candidates for *in-situ* mycoremediation.

Materials & Methods

Study Area

The study area is located at Barangay Balili, Mankayan, Benguet (16°51'38.96" N 120°51'15.07" E; elevation: 2315 masl) (Fig. 1). The farm is regularly planted with potato (*Solanum tuberosum*) in the area and that the plants are sprayed with a combination of the insecticide Cypermethrin and fungicide Mancozeb.

Soil Sample Collection

Soil collection was according to the methods of Harish et al. (2013). At a random area within the plantation, soil sample was obtained from the upper surface area of 0-15 cm soil layer of the garden then stored in an airtight polyethylene bag. The bag was kept at 4°C in an ice box and was transported immediately to the laboratory of the University of the Philippines Baguio.

Preparation of Pure Fungal Cultures

The procedure for culturing fungi was adopted from Magnuson & Lasure (2002). Ten percent soil suspension was prepared in a solution of 10 mM potassium phosphate, 150 mM NaCl, and 0.02% SDS (PBS-SDS), at pH 7. The suspension was agitated at 130 rpm in 250 mL Erlenmeyer flasks for 20 minutes. Next, the soil particles were left to settle for 15 minutes before the liquid was decanted.

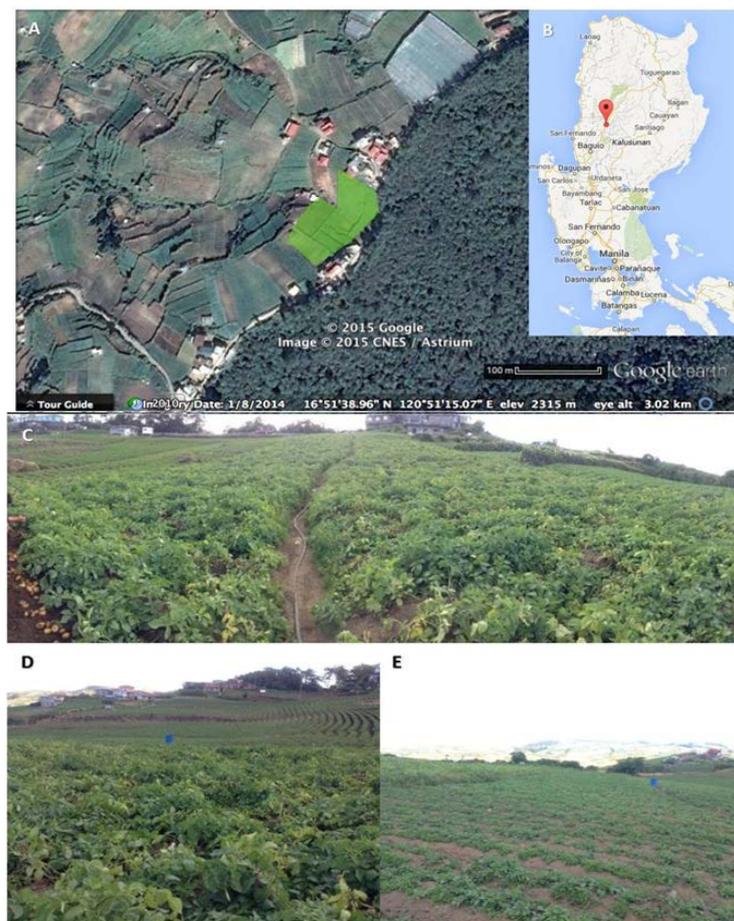


Fig. 1 – (A) A satellite image of the potato plantation. Image taken from Google Earth, 2015. (B) Map of Luzon Island, Philippines, showing the location of the plantation relative to Manila. Image taken from Google Maps, 2015. (C) The potato plantation, showing the cultivated area. Shading is noticeably absent. (D). Edges of the plantation populated by trees and houses. (E) An area within the plantation carved into a mountainside.

The decantate was then serially diluted to up to 1:1000 in PBS. The diluted soil extracts were spread on plates of Potato Dextrose Agar (PDA) with chloramphenicol (200 µg/ml) and rose bengal (200 µg/ml), and were incubated at 30°C, until colonies of at least 5 mm have grown.

Colonies in the plates that have unique morphological characteristics were taken as a separate isolate. Characteristics that were considered include any combination of the following: colony size, elevation, margin, surface texture, opacity, and color or pigmentation. Each isolate reinoculated in a PDA slant that contained chloramphenicol (200 µg/ml) and was incubated at 30°C for 7 days.

Morphological Analysis of Filamentous Soil Fungi

The morphological studies were conducted using macroscopic and microscopic analysis of fungal colonies.

Pure cultures of each filamentous soil fungi were sub-cultured in petri plates containing PDA media with chloramphenicol (200 µg/ml). They were incubated at 30°C for 5 days. The grown mycelia were studied under a Swift® SM100 Series Stereozoom microscope for the macroscopic analysis. The macroscopic features observed were size, elevation, margin, surface texture, opacity, and color.

Slide cultures were prepared for the microscopic analysis of the hyphae and spores. A block of PDA was transferred aseptically to the center of a sterile glass slide contained in a sterile petri plate. The four sides of the agar block were inoculated with spores or small portions of the mycelia. A sterile cover slip was laid over the agar block. The cultures were incubated for 3 days. Lactophenol methyl blue was used to stain the slide cultures.

Prepared slides were then pictured under an OPTIKA B-150 binocular electric microscope under high power objective.

Molecular identification of Filamentous Soil Fungi

DNA was extracted from the isolates using the Rapid Mini-Preparation of Fungal DNA for Polymerase Chain Reaction (PCR) by Liu et al. (2000).

PCR was conducted to amplify the D1/D2 regions of the large subunit of the 28S rDNA. Twenty five µl of EmeraldAmp GT PCR Master Mix, 0.5 µl Reverse Primer (NL-1), 0.5 µl Forward Primer (NL-4), and 23 µl PCR grade water were added to PCR tubes containing 1 µl of the DNA template obtained from DNA extraction of each pure culture. PCR tubes were run in LabNet® Multigene Gradient PCR machine for 3 hours. The products of PCR and the DNA were run through gel electrophoresis. Appearance of bands confirmed presence of DNA sequences. These were then sent to 1st BASE, Singapore for polymerase chain reaction and DNA Sequencing of target rDNA.

DNA sequences were submitted to Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) Genbank (blast.ncbi.nlm.nih.gov) for identification. Resulting DNA sequences showing ≥98% similarity were considered to belong to the same species (Arnold & Lutzoni 2007) while sequences showing ≥95% similarity were considered to belong to the same genus. Moreover, sequences that showed <95% similarity were considered unidentified taxon (Sánchez-Márquez et al. 2007).

Carboxylesterase Activity Assay

This test for carboxylesterase activity in fungi was carried out according to Avicor et al. (2012) where the enzyme acts on 1-naphthyl acetate and releases 1-naphthol with slight modifications. Three replicates were done for each isolate. Approximately, a 5 x 5 mm mycelia was aseptically cut from a plate culture and placed into a test tube containing 2.8 ml of phosphate buffer solution (pH 7.0). A control set-up was also made that does not contain fungi. A 30 mM 1-naphthyl acetate solution was prepared by dissolving Sigma® 1-naphthyl acetate in acetone. Next, a 100 µl of the 30 mM 1-naphthyl acetate solution was pipetted into the test tubes. Then, the mixture was incubated for 10 minutes in at 40°C in a water bath.

After incubation, the reaction was stopped by adding 0.25 ml of stop solution. The stop solution was adapted from Sree & Varma (2015), which consisted of 3.4% sodium dodecyl sulphate along with 0.8% Sigma-Aldrich® Fast Blue B salt, dissolved in Type 1 water. The Fast Blue B dye formed a complex with 1-naphthol that resulted in a change in absorbance. Prior to spectrophotometric analysis, the Shimadzu UVmini-1240 UV-Vis Spectrophotometer was tared using type 1 water as a blank. The absorbance of the resultant mixture was read immediately after adding the stop solution at 600 nm. An increase in the absorbance of the sample against the control indicates carboxylesterase activity. The readings for each isolate were averaged. Carboxylesterase activity was then calculated using a calibration curve prepared from a standard solution of 36µg/ml of Sigma-Aldrich® 1-naphthol and its 1:1 serial dilutions (Mohammed 2010). Resultant absorbance readings were plotted against the corresponding 1-naphthol standard curve.

Statistical Analysis of Assay Results

Statistical analysis was carried out using IBM SPSS version 21 using One-Way ANOVA ($p < 0.05$). A Post hoc range test was done using Ryan-Einot-Gabriel-Welsch Q (R-E-G-W Q) post hoc criterion.

Results

Filamentous Soil Fungi Identification

Fourteen morphologically unique filamentous fungal isolates were taken from the soil dilution plates. The macroscopic morphological characteristics of the colonies were viewed under the Stereozoom microscope (Fig. 2). The following characteristics were observed in the macroscopic analysis: mycelia elevation, margin, surface, opacity, and color (Table 1). Convex, flat, nimbonate and raised were the types of elevation observed. One isolate exhibited the nimbonate type, two were convex, five were raised and six were flat. The most observed type of margin is filiform wherein 11 isolates exhibited it followed by the entire type which was exhibited by two fungal isolates. Only one had the lobate type. The glistening type of mycelia surface was the most occurring as seven isolates exhibited it. Four had a rough surface and three exhibited the dull type. In terms of opacity, all isolates were opaque.

On the other hand, the microscopic morphological characteristics of the hypha and spores of the isolates were observed under an electric microscope (Fig. 3). All of the fungal isolates exhibited a septate hyphae which indicated that they belong to Division Ascomycota. Three different types of spores were noted. These were the microconidia exhibited by five fungal isolates, the complex conidia exhibited by three, and the chlamyospore exhibited by three fungal isolates. Only BDP 7 exhibited two types of spores in its mycelia in the form of a microconidia and chlamyospore. The spores of the four isolates were not determined since no spores were formed in the cultures.

Eight of the fourteen isolates were identified using a query search in GenBank® using their DNA sequences from 1st BASE. The identified species were *Fusarium oxysporum*, *Fusarium* sp. *Aspergillus heteromorphus*, and *Penicillium* sp. It was observed that the gene sequences of five isolates (BDP5, BDP6, BDP7, BDP11, and BDP14) correspond to one species match, *F. oxysporum*, but were found to be of different strains (Table 2). All other isolates were determined by the authors as unidentified as their maximum identity values were lower than 98%.

Carboxylesterase Activity Assay

Absorbance values of each fungal preparation at 600nm were extrapolated against a 1-naphthol calibration curve ($y = 0.0416x + 0.0569$, $R^2 = 0.9937$) in order to get the 1-naphthol equivalent concentration in each preparation. Post hoc analyses using R-G-E-W Q range test created homogeneous subsets among the isolates. It confirmed that the control has a significantly lower 1-naphthol equivalent concentration than all the isolates (Fig. 4). In addition, BDP 3 and BDP 10 were grouped into a single subset that the highest 1-naphthol equivalents than all other isolates. The others grouped together with intermediate 1-naphthol equivalents.

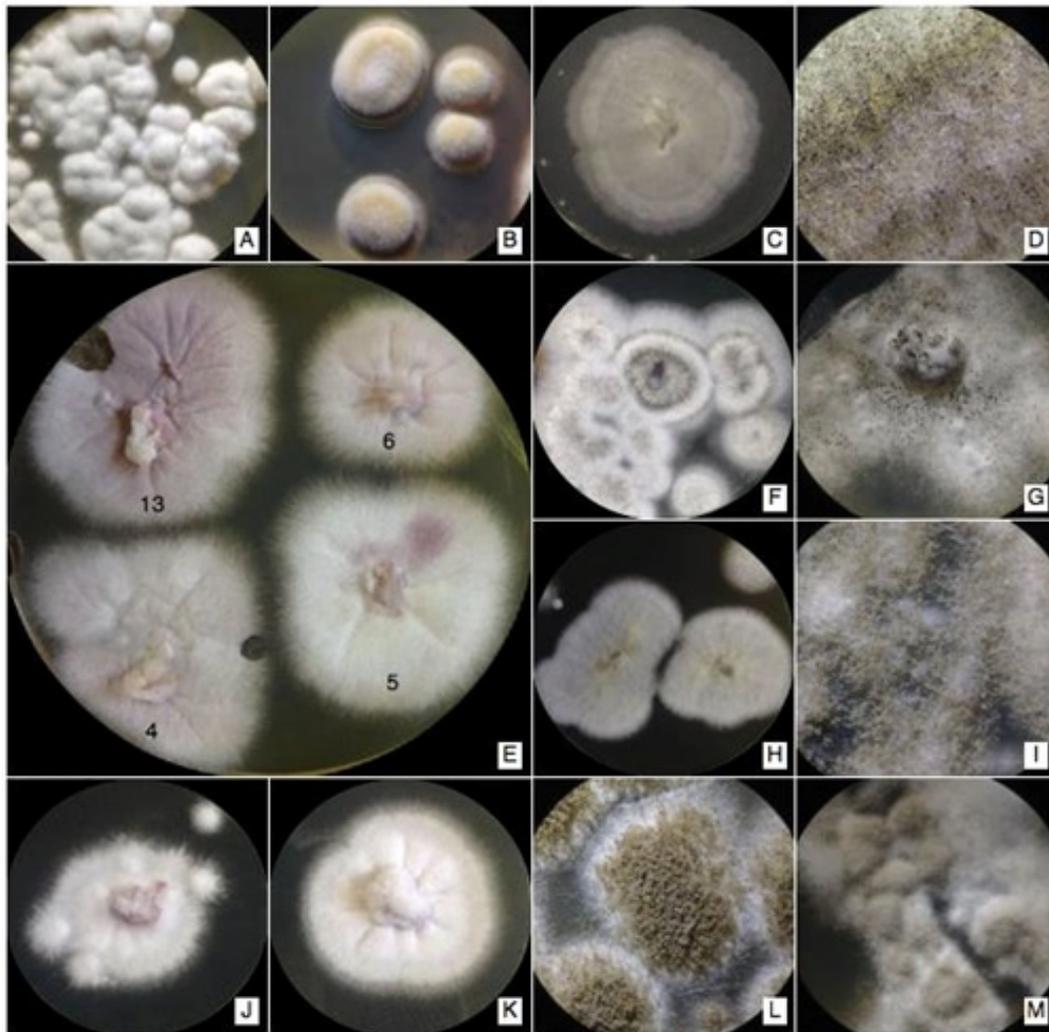


Fig. 2 – Filamentous fungal isolates from the soil of a potato plantation in Mankayan, Benguet. (A) BDP1. (B) BDP2. (C) BDP3 (D) BDP3. (E) *Fusarium* isolates. Clockwise from top left: *Fusarium* sp., *F. oxysporum* f. sp. *lycopersici*, *F.oxysporum*, *Fusarium* sp. (F) *Aspergillus heteromorphus*. (G) *A. heteromorphus*. (H) BDP9. (I) BDP9. (J) *F. oxysporum* strain FOCCB. (K) *F. oxysporum*. (L) BDP10. (M) *Penicillium* sp. A, B, D, G, I, L, and M were viewed under stereomicroscope.

Table 1 Morphological characteristics of filamentous fungi isolated from the soil sample taken from a potato plantation in Mankayan, Benguet.

Isolate	Macroscopic						Microscopic	
	Diameter	Elevation	Margin	Surface	Opacity	Color	Septa	Spore
BDP1	4 mm	convex	entire	dull	opaque	white to brown	septate	NSF*
BDP2	8 mm	convex	entire	dull	opaque	brick red to cream	septate	NSF*
BDP3	10 mm	flat	lobate	rough	opaque	white hypha, black spores	septate	chlamy-dospore
BDP4	14 mm	flat	filiform	glistening	opaque	pink	septate	micro-conidia
BDP5	7 mm	raised	filiform	glistening	opaque	white	septate	NSF*
BDP6	18 mm	raised	filiform	glistening	opaque	purplish white	septate	micro-conidia
BDP7	1 mm	raised	filiform	glistening	opaque	pinkish white	septate	micro-conidia,
BDP8	11 mm	flat	filiform	rough	opaque	white hypha, dark brown spores	septate	complex conidia

Table 1 Continued.

Isolate	Macroscopic						Microscopic	
	Diameter	Elevation	Margin	Surface	Opacity	Color	Septa	Spore
BDP9	8 mm	flat	filiform	Rough	opaque	white hypha, yellow green spores	septate	chlamy-dospore
BDP10	10 mm	flat	filiform	rough	opaque	white hypha , green spores	septate	complex conidia
BDP11	15 mm	raised	filiform	glistening	opaque	purplish white	septate	NSF*
BDP12	35 mm	nimbonate	filiform	dull	opaque	brown	septate	complex conidia
BDP13	15 mm	raised	filiform	glistening	opaque	purplish white	septate	micro-conidia
BDP14	10 mm	flat	filiform	glistening	opaque	white	septate	micro-conidia

*No spores found

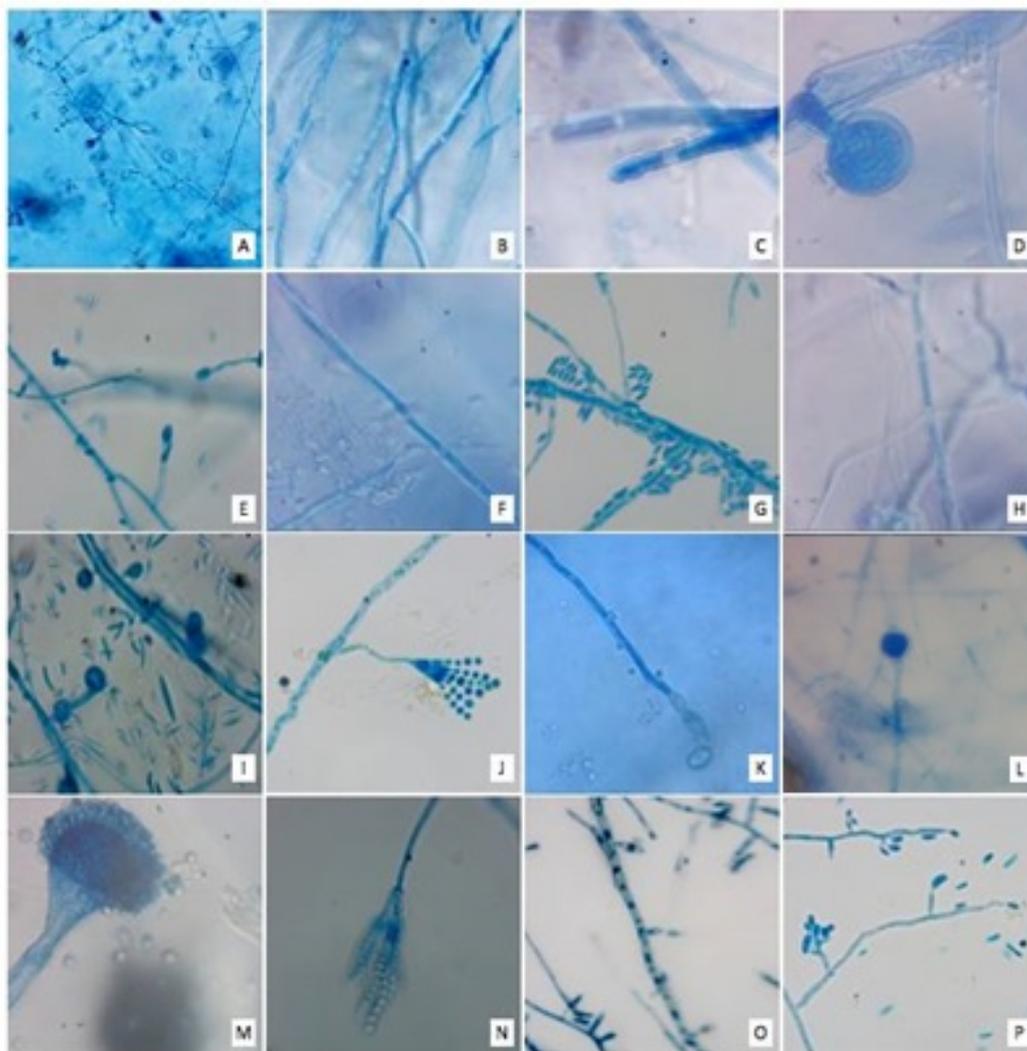


Fig. 3 – Filamentous fungal isolates from the soil of a potato plantation in Mankayan, Benguet. Stained with lactophenol methyl blue. Viewed under 400X magnification. (A) BDP1, branching hypha. (B) BDP2, hypha. (C) BDP3, budding hypha. (D) BDP4, chlamydospore. (E) *Fusarium* sp., hyphae. (F) *F. oxysporum*, hyphae. (G) *F. oxysporum* f. sp. *lycopersici*, microconidia. (H) *F. oxysporum* f. sp. *lycopersici*, hypha. (I) *F. oxysporum* strain FOCCB, microconidia. (J) *Aspergillus heteromorphus* strain SF-6390, complex conidia. (K) BDP9, hypha. (L) BDP9, chlamydospore. (M) BDP10, complex conidia. (N) *Penicillium* sp., complex conidia. (O). *Fusarium* sp., microconidia. (P) *F. oxysporum*, microconidia.

Table 2 GenBank query search results and identification of the filamentous fungi isolated from soil taken from a potato plantation in Mankayan, Benguet.

Isolate	Nearest Match	Query Cover	E Value	Max Identity	Identification
BDP1	<i>Penicillium gerundense</i> (EU427291.1)	93%	3e-54	70%	Unidentified Ascomycete
BDP2	<i>Aspergillus sylvaticus</i> (EF652448.1)	92%	4e-148	83%	Unidentified Ascomycete
BDP3	<i>Aspergillus deflectus</i> (EF652437.1)	75%	9e-24	68%	Unidentified Ascomycete
BDP4	<i>Fusarium cf. oxysporum</i> OP 12.d(KP017399.1)	97%	0.0	99%	<i>Fusarium</i> sp.
BDP5	<i>Fusarium oxysporum</i> (EF363781.1)	97%	0.0	99%	<i>Fusarium oxysporum</i>
BDP6	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (EU214564.1)	91%	0.0	99%	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>
BDP7	<i>Fusarium oxysporum</i> (EF363781.1)	97%	0.0	98%	<i>Fusarium oxysporum</i>
BDP8	<i>Aspergillus heteromorphus</i> strain SF-6390 (KM434330.1)	96%	0.0	99%	<i>Aspergillus heteromorphus</i> strain SF-6390
BDP9	<i>Fusarium oxysporum</i> (EF363781.1)	96%	0.0	87%	Unidentified Ascomycete
BDP10	<i>Penicillium janthinellum</i> (HM469429.1)	89%	1e-129	82%	Unidentified Ascomycete
BDP11	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (EU214564.1)	91%	0.0	99%	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>
BDP12	<i>Penicillium</i> sp. (GQ169751.1)	95%	0.0	98%	<i>Penicillium</i> sp.
BDP13	<i>Fusarium oxysporum</i> (KJ126550.1)	95%	0.0	97%	<i>Fusarium</i> sp.
BDP14	<i>Fusarium oxysporum</i> (EF363781.1)	97%	0.0	99%	<i>Fusarium oxysporum</i>

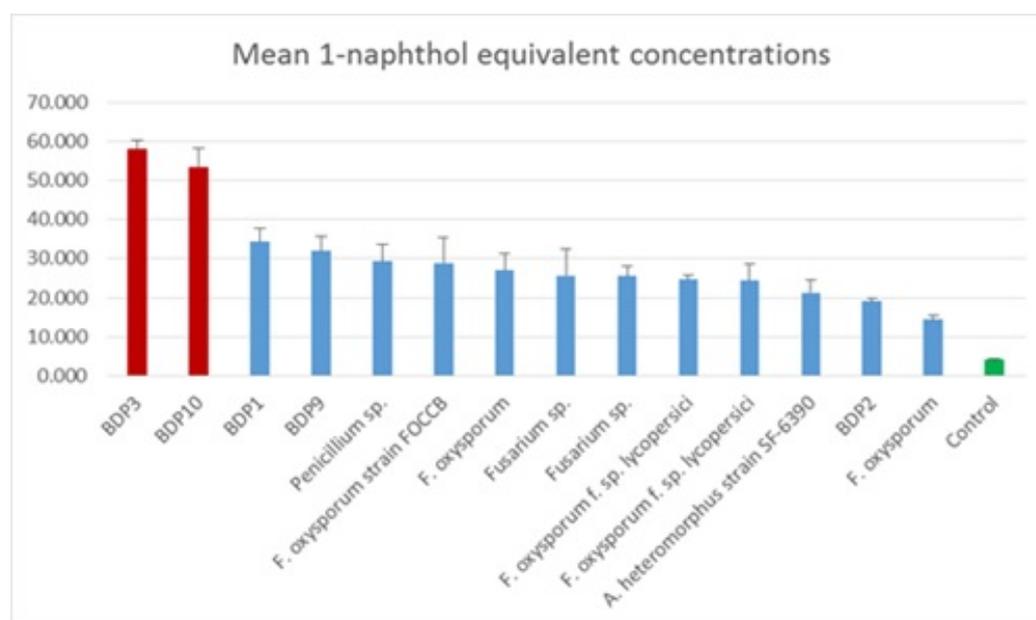


Fig. 4 – Mean concentration of 1-naphthol equivalent in the assay preparations for each fungal isolate. Means were grouped into subsets at $p=0.05$ using the R-G-E-W Q post hoc criterion. All isolates exhibit significantly higher carboxylesterase activity than the control. Isolates BDP3 and BDP10 were separated into a single subset that exhibited the highest carboxylesterase activity.

Discussion

Fourteen isolates with unique morphological characteristics were collected from a soil suspension of the soil collected in Mankayan, Benguet. All of the isolates were sequenced and identified to be members of the Ascomycetes. This is expected because of the wide distribution of this group of fungi.

There seems to be low diversity among the isolates. Despite having exhibited differences in their morphology, especially in their color, seven of the isolates were identified to be *Fusarium*, five of which were identified at species level as *Fusarium oxysporum*. This species belongs to Class Sordariomycetes. The two other isolates identified using sequencing, *Aspergillus heteromorphus* and *Penicillium* sp., are closely related species (Samson et al. 2014). Both of them belong to Class Eurotiomycetes.

Fusarium is a ubiquitous species (Saika & Kadoo 2010). They are well represented in any type of soil worldwide (Burgess 1981). The varying morphologies of the fungi may be due to them belonging to different strains despite them being similar species. The occurrence of *F. oxysporum* species in the isolates might be due to the abundance of host plants (potatoes) in the plantation from which they were isolated. The said species are known pathogens of plants regardless of their strains. In the study of Manici & Cerato (1994) and Bayona et al. (2011), *F. oxysporum* isolates from infected tubers caused dry rot on inoculated tubers and infected the vascular tissues through roots and leaves.

Species belonging to the genus *Aspergillus* and *Penicillium* are ubiquitous soil inhabitants (Keller & Bidochka 1998). The isolation of *Penicillium* sp. and *Aspergillus heteromorphus* from the plantation suggests that the fungal species play a role *in-situ*. In the research conducted by Salami & Popoola (2007), they isolated a *Penicillium* species in a diseased potato associated with post-harvest rot. Some *Penicillium* spp. isolates from the soil therefore could either be parasitic or saprophytic. On the other hand, no literature exists that indicate that *A. heteromorphus* is pathogenic to plants. Research journals instead indicate that they are capable of producing laccases (Singh et al. 2010) and cellulases (Singh et al. 2009) to degrade organic biomass. Hence, suggesting that the fungal isolate is saprophytic.

Results from the 1-naphthol assay test indicate that all were able to degrade cypermethrin thus indicating carboxylesterase activity. This ability may be attributed to their chronic exposure to pyrethroids *in-situ* (Afify et al. 2013). Considering that the isolates were extracted from soil where the pyrethroid chemical – cypermethrin, was regularly applied, the isolates may have already adapted to such conditions. Thus it is possible that the pesticides applied in the garden are sources of carbon and nitrogen upon degradation by the isolates (Ellegaard-Jensen 2012).

Indication of carboxylesterase activity would imply that all have the potential to degrade pyrethroids such as cypermethrin and organophosphate pesticides that contain xenobiotic ester compounds *in-situ* (Wang et al. 2009). It is, however, important to consider the ecological roles of these fungi in order to be considered as viable candidates for mycoremediation. *Fusarium*, being known parasites, cannot be considered despite exhibiting carboxylesterase activity because these fungi may infect crops and reduce yield as it degrades the pesticide residues. The promising candidates for use in mycoremediation are isolates BDP3 and BDP10. Both are unidentified species but are more likely to be related to *Aspergillus* or *Penicillium* based on their morphology and on their close homology based on their D1/D2 sequence. Post hoc analysis results show that these isolates have exhibited the highest carboxylesterase activity among all isolates. The result suggests that the two isolates are more efficient at producing the carboxylesterase enzyme. Results therefore suggest that these would perform best at degrading cypermethrin and other pyrethroids and thus are prime candidates for mycoremediation of pyrethroid pollution *in-situ*.

Conclusion

A total of 14 isolates were isolated from the soil sample collected from a potato plantation in Mankayan, Benguet. Nine isolates were identified using morphological analysis and sequencing of the D1/D2 regions of the 28S rDNA. Of these nine isolates, seven belong to the genus *Fusarium*,

five of which were identified to be *F. oxysporum*. The other two isolates were identified to be *Aspergillus heteromorphus* and *Penicillium* sp. In the 1-naphthol assay, it was found out that all of the isolates exhibited carboxylesterase activity. Post hoc analysis of the results showed that two unidentified Ascomycetes – BDP3 and BDP10, exhibited the greatest carboxylesterase activity. These two isolates are promising species for mycoremediation, specifically targeting pyrethroid pesticides.

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