

RESEARCH ARTICLE

Investigation of possible vesicular arbuscular mycorrhizal associations in prevalent weeds in tea lands of Badulla

D.H.Y.A. Ranasinghe*, K.G. Prematilake, P.D.P.M.D. Silva, H.M.P.M. Gunasena

Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellasa University, Badulla, 90000, Sri Lanka

Submitted: May 21, 2020; Revised: May 10, 2021; Accepted: May 30, 2021

*Correspondence: vasassriranasinghe@gmail.com

ABSTRACT

A study was carried out to investigate the possible vesicular arbuscular mycorrhizal (VAM) associations in prevalent weeds in selected tea lands of Badulla. Roots of *Ageratum conyzoides*, *Axonopus compressus*, *Bidens pilosa*, *Borreria latifolia*, *Cleome rutidosperma*, *Drymaria cordata*, *Eleusine indica*, *Erigeron sumatrensis* and *Oxalis corniculata* were collected from Wewessa, Spring Valley and Telbedde estates. Colonization percentages and spore counts were calculated using the Grid and Doncaster's counting disc methods, respectively. Rhizosphere soil of highly VAM associated weeds were tested for soil phosphorous and all weeds were tested for soil pH. The highest colonization percentage was recorded as 60.39% with *A. compressus*. The highest spore number was counted as 187 per 5 g soil with *B. latifolia*. The lowest soil phosphorus level was measured as 4.17 ppm with *A. conyzoides*. There was a systematic moderate positive correlation between, root colonization percentages and soil pH. In conclusion, there was a close association of VAM with selected nine weed species.

Keywords: Vesicular arbuscular mycorrhizae (VAM), root colonization, VAM spore count, weeds, soil phosphorus

INTRODUCTION

Weeds are plants which grow out of place or growing where it is not wanted (Blatchley, 1912). When weeds present in any land, it could interfere with the productivity and growth of crop by competing with space, sun light, nutrient and water. Although, weeds are condemned weeds do play some hidden beneficial roles too.

One of them is VAM association (De Silva, 2016). VAM are endomycorrhizae that grow in root cortex forming specific fungal structures, referred to as vesicles and arbuscules. This characteristic growth gives endomycorrhizae the alternate name, vesicular arbuscular mycorrhizae (Sieverding, 1991). The importance is, it has proven that VAM is facilitated to improve the productivity in the low fertility soil (Jeffries, 1987) in terms of increasing the uptake of slowly diffusing ions such as phosphate (PO_4^{3-}) (Jacobsen *et al.*, 1992), immobile nutrients such as phosphorus (P), zinc (Zn) and copper (Cu) (Lambert *et al.*, 1979; George *et al.*, 1994; George *et al.*, 1996; Ortas *et al.*, 1996; Liu *et al.*, 2002; Quilambo,

2003) and other nutrients such as cadmium (Cd) (Guo *et al.*, 1996). Under drought conditions, the uptake of highly mobile nutrients such as nitrate (NO₃⁻) can also be enhanced by mycorrhizal associations (Azcon *et al.*, 1996; Subramanian and Charest, 1999; Quilambo, 2003) etc. Further, Jordan *et al.* (2000) suggested that mycorrhizae can change the function of weed communities as the net effect of weeds becomes more beneficial to crops which would more possible if weeds promote the growth of mycorrhizae that later colonize in the rhizosphere of the crop. This explains why some crops grow better following some weed communities (Ilangamudalil and Senarathnel, 2016).

Fortunately, about 80% of all terrestrial plant species including crops and weeds belong to families which are characteristically mycorrhizal (Smith and Read, 1997). According to the previous studies such as arbuscular mycorrhizal fungi in different land types at Upper Hantana, (Mafaziyal and Madawala, 2015); Mycotrophy in some tropical weeds in Forest Research Institute in Dehradun, Uttarakhand State of India (Chawla *et al.*, 2011); Arbuscular mycorrhizal morphology in associated weeds in tropical agro-ecosystems at agricultural fields in Coimbatore (Muthukumar and Prakash, 2009); Mycorrhizal associated weeds in tea estate at Assam Agriculture University Jorhat (Hazarika, 2000); Arbuscular mycorrhizal fungi: potential roles in weed management (Jordan *et al.*, 2000); Mycorrhizae and root-associated fungi in Spitsbergen (Viire *et al.*, 1992); scientists have recognized some VAM associated weeds with their colonization percentages and spore counts.

Further, due to the ban of some weedicides and lack of labour in tea estates and tea lands are prominent with weeds. Thus, there is a possibility for VAM occurrence in such lands. Therefore, the investigation of associations of VAM in the rhizosphere which makes mutualistic symbiosis (non-pathogenic association) with some prevalent weeds in tea lands is essential. Accordingly, this study has focused on root colonization of VAM and prevalence of VAM spores in the rhizosphere with respect to selected weeds. Further, the study focused on the availability of soil P in higher VAM associated weeds and the relationship between colonization percentage or spore count and soil pH levels.

If the weeds under this study are found to be highly VAM associated, that land can be recommended as a sustainable one as they can play an important role in maintaining mycorrhizal inoculum in the soil, while enhancing the soil nutrient availability. Also, this study would be helpful to introduce new ecological and biological methods for the maintenance of tea lands and reduce the cost of weeding. For an example, as VAM associated weeds are less or not competitive with the tea crop, it could be possible to leave the weeds' shallow root system/root ball in the crop field by practising slash weeding in order to less reduction or retention from tea lands. Further, it can practice in keeping the VAM associated soft unavailing weeds for live mulch to cover the ground while conserving the biodiversity. Thus, for further recommendations also weeds can

be investigated for their potentiality of VAM association (non-pathogenic association).

Therefore, this study was initiated to identify the VAM associated weeds and their magnitude of VAM association, especially relative to the tea lands in IM_{1a} agro-ecological zone of Badulla district in Uva region as this area is highly considered for tea.

MATERIALS AND METHODS

This study was conducted with randomly selected nine non-problematic weeds/soft herbs (*Ageratum conyzoides*, *Axonopus compressus*, *Bidens pilosa*, *Borreria latifolia*, *Cleome rutidosperma*, *Drymaria cordata*, *Eleusine indica*, *Erigeron sumatrensis* and *Oxalis corniculata*) during the month from October to December 2019, covering three tea estates (nearest to the studied location in IM_{1a} agro-ecological zone of Badulla district); Wewesse, Spring-Valley and Telbedde of the Balangoda Plantations PLC where the elevations above sea level are around 1168 m, 1148 m and 853 m, respectively (Lk. geoview.info., 2021). In consideration with the soil properties in this area nearly the depth is 80.8 cm, gravel percentage is 35.4%, sand percentage is 53.6%, bulk density is 1.18 gcm⁻³ and organic carbon (OC) percentage is 2.17% (Wijeratne and Chandrapala, 2014). The region receives an annual rainfall of about 2,000 mm and average temperature lies between 20 to 25 °C (Road Development Authority, Ministry of Higher Education and Highways for the Government of Sri Lanka and the Asian Development Bank, 2017).

According to the De Silva *et al.* (2003), morphological characteristics of nine weed species were studied. In sample collection top (1-2 cm) surface litter layer was scraped away and each weed was carefully pulled out. Roots together with rhizosphere soil adhere to roots from a depth of 2-15 cm were collected into a sterile polyethene bag and stored at 4 °C for further analysis (Chawla *et al.*, 2011). Ten samples of each nine weed species were separately obtained from both cultivated and barren lands of each 3 estates. Then, three composite samples were made out of both land types in all estates. These samples were used to investigating the root colonization, spore population, soil P analysis and soil pH.

Roots were stained by acid Fuschin stain method (Phillips and Hayman, 1970). There, 3 g of composite fresh rinsed roots from each weed were cut into approximately 1 cm length. Distilled water, 2.5% aqueous solution of KOH (w/v), 1% HCl and Acidic Glycerol solution containing 0.05% Trypan Blue were used for staining. Using a compound microscope VAM colonies were observed. The grid line intersect method was used to quantification (Giovannetti and Mosse, 1980). There, 5 root parts from each sample were assessed. Using the following formula, root colonization percentage was calculated.

$$\text{Colonization (\%)} = (A_1/A_2) \times 100$$

A₁ – Number of intersects with VAM

A₂ – Total number of intersects

Wet sieving and decanting technique (Gerdemann and Nicholson, 1963) were used to separate organic debris from sieve (1000, 150 and 125 µm sieves) and air dried. 5 g of soil from each sample was separately placed in a 15 mL centrifuge tube, filled with distilled water and centrifuged under 2,500 rpm for 10 to 15 min. Sugar density gradient centrifugation method (Daniel and Skipper, 1982) was used for the extraction of VAM spores. There, 2 M sucrose-NaCl solution was used to refill the tubes and centrifuged for 20 min at 2,500 rpm. The sucrose-NaCl solution containing spores, representing each weed sample was poured over the separate Whatman® filter paper. VAM spores were counted by Doncaster's counting disc method (Brundrett, 2008). There, under the dissecting microscope, VAM spores available in the divided portions were alternatively counted covering 8 divided portions of the filter paper. The number of spores to be availed in 16 portions were calculated.

For the soil P analysis Olsen method (Olsen, 1988) was followed. As the colouring agent p-Nitrophenol was used. The absorbance of 1 g soil sample was measured at 660 nm in a UV-VIS Spectrometer against a blank (distilled water). The concentration of samples was calculated using the equation based on the calibration curve: where 'y' was the absorbance and 'x' was the concentration of P standard solution.

Under 19±1 °C temperature using a pH meter, pH of 1:3 soil medium- distilled water suspension was measured.

As a secondary data collection data on fertilizer applications were collated from three estates for six months' period before sample collection. Simple calculation was done to record the applied P levels in ppm.

Two-way MANOVA (Multivariate) was performed for root colonization percentage and spore count analysis. Two-way ANOVA (Univariate) was performed for soil P level analysis. There, secondary data was used as covariate to analyse root colonization percentage, spore count and soil P level. Descriptive statistics was used to estimate the mean soil pH; further, correlation was performed to evaluate the variate the colonization and spore population with the soil pH level. IBM SPSS 23 Software was used (Pallant, 2005). The significance of the differences between treatments was tested at 0.05 significant level.

There, the three estates were considered as blocks. Two land types (cultivated land as experimental plot and barren land as control plot) from each block were considered as the main treatments, nine weed species were considered as sub treatments and the mycorrhizal association status was characterized by the

presence of vesicles and spores only [vesicles as colonization percentage, spores as spore count (number of spores per 5 g soil)]. Applied P levels (ppm) from secondary data were considered as the covariates.

RESULTS AND DISCUSSION

The overall mean colonization percentage and spore count were reported as 49.59% and 155.43 per 5 g soil, respectively. Results indicated that VAM association in three estates were significantly ($P<0.01$) varied. Meanwhile, the difference between cultivated land and barren land was significant only at $P<0.1$ and different weed species were significantly varied at $P<0.01$ level. Further, colonization percentage and spore count of three estates were highly significant. Both colonization percentage ($P<0.05$) and spore count ($P<0.01$) were significantly different in nine weed species as well whereas only the colonization percentage has been significantly varied ($P<0.05$) in two different land types.

VAM associated weeds were reported earlier by many researchers such as, Singh and Verma (1981), Barthakur and Arnold (1989), Rathi and Singh (1990) and Chawla *et al.* (2011) under different ecosystems. There, a significant relationship was mainly developed from the physical expression of symbiosis in terms of root colonization and soil spore population (Ilangamudali and Senarathnel, 2016). However, the majority of the fungal mycelia and the associated structures (vesicles, arbuscules and spores) were found within the absorptive zone of individual roots which are very often concentrated in the inner cortex of feeder roots (Butler, 1938; Balasuriya *et al.*, 1991). Present study has also supported to prove that all selected nine weed species are VAM associated that had vesicles in their root cortex and spores in the rhizosphere.

In addition, different weeds were supported for different mycorrhizal colonization percentages in their root systems and different spore population in the rhizosphere. St. John (1980) had found that trees of the order Asterales were heavily mycorrhizal and Brundrett (2009) found that plants of the family Caryophyllaceae to which *S. media* belongs were non-mycorrhizal. According to this study, Asteraceae, Poaceae, Capparaceae, Rubiaceae and Oxalidaceae families are found to be mycorrhizal.

Fungi forming mycorrhizal associations with roots of perennial plants are not free from VAM interaction (Balasuriya *et al.*, 1991). This study has further supported to prove that perennials are VAM associated. However, from previous studies by Mafaziyal and Madawala (2015), Chawla *et al.* (2011) and Hazarika (2000) including this study has proven that annuals can also be defined under VAM associated plants. Furthermore, this study has also supported to figure out both grass species (*Axonopus* spp. and *Eleusine* spp.) and herbaceous species which are under annuals also found to be VAM associated.

When considering the estates, lands and weed separately, the results were reported as follows. Mean colonization percentages of Wewesse, Spring Valley and Telbedde estates have been reported as 54.67, 45.92 and 48.18%, respectively. Only Wewesse and Spring Valley have been reported a mean difference of 8.75% which is significant at $P < 0.05$. Whereas, there was no any significant difference in colonization between Telbedde estates with other two. However, the mean difference between Wewesse and Telbedde was 6.49% and between Spring Valley and Telbedde was 2.26%. Mean spore count of Wewesse, Spring-Valley and Telbedde estates have been reported as 81.5, 166.5 and 139 per 5 g soil, respectively. Wewesse and Spring Valley have reported a mean difference of 85.1 per 5 g soil which was significant at $P < 0.01$. Wewesse and Telbedde has reported a mean difference of 58.0 per 5 g soil which was significant at $P < 0.01$. However, there was no significant ($P > 0.05$) difference between Spring Valley and Telbedde though the difference between two estates was 26.7 per 5 g soil.

According to the Chawla *et al.* (2011), mycorrhizal status within different sites have no definite pattern of root infection emerged although, they were statistically significant. In that study, this was revealed by weeds at Howard Road which had the lowest root colonization (61.3%) whereas weeds at Pearson Road had the highest root colonization (70.0%). This has been further supported by the present study that colonization percentages and spore count between some estates have been reported significant differences.

The mean colonization percentages under cultivated and barren land have reported as 41.9 and 57.29%, respectively, with a significant difference of 15.39% at $P < 0.05$.

In contrast to mean colonization percentage, the mean spore counts of cultivated and barren land have been reported other way round as 135 and 114 per 5 g soil, respectively. However, the difference (21 per 5 g soil) was not reported as significant even at $P < 0.1$. The pattern of mean root colonization is further described with different weeds under two land types as shown in Figure 1. Mean colonization percentages in weed spices have always been reported higher in barren land compared to the cultivated land.

Mean spore count (Figure 2) was higher in cultivated land compared to that of barren lands. However, the mean spore count result was only because of *Borreria* spp. and *Erigeron* spp. whereas in all other weeds, it was other way round. Therefore, the higher mean spore count in cultivated land was mainly attributed to the presence of higher spore count with *Borreria* spp. and *Erigeron* spp.

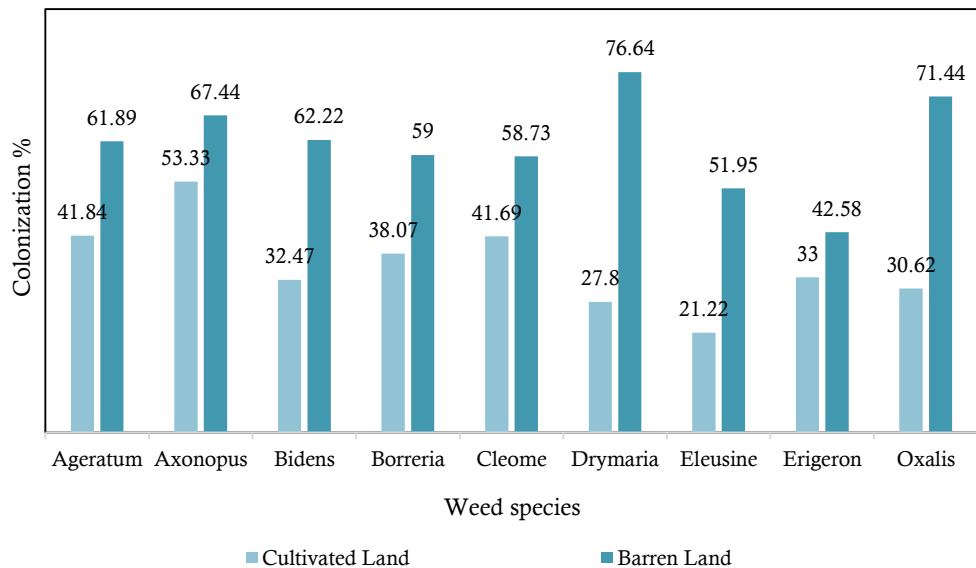


Figure 1: Mean colonization percentage reported with different weed species in two lands types.

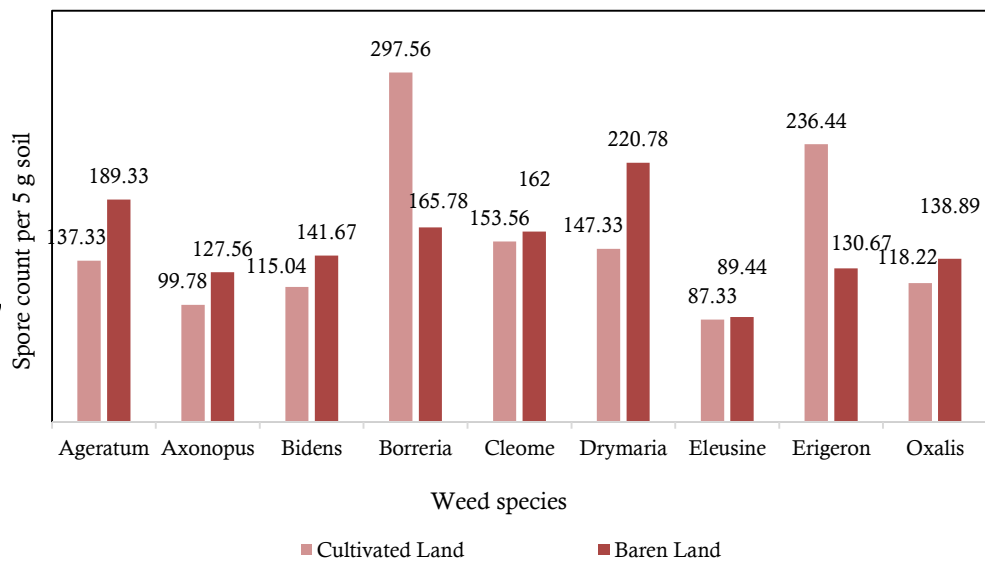


Figure 2: Mean spore count reported with different weed species in two land types.

Previous studies showed that a higher level of P fertilization has resulted in a suppressive effect of fungal colonization leading to malformed or arbuscules (Breuillin *et al.*, 2010). It is further supported by the measurements of higher colonization percentage in barren land than in cultivated lands in the present study. However, one recent study has found that investment (as a whole) in storage vesicles increased four-fold in fertilized compared to the control plots but this might be due to the shifts in species composition inside roots or changes in allocation strategy was unknown (Olsson, 2010). VAM species such as *Glomus intraradices* (Verbruggen and Kiers, 2010) is known to be tolerated high P levels and from the fungal point of view, allocation of more carbon to storage is vice when nutrients are abundant (Breuillin *et al.*, 2010). Similarly, higher spore population of *Borreria* spp. and *Erigeron* spp. in cultivated land of this study may also be due to these circumstances. Further, suspicious of lesser spore population of *Borreria* spp. and *Erigeron* spp. in barren land in comparison to the cultivated land are that, the lesser abandon time period was not be enough to considered as a barren land (control plot) for this study and run-off in non-prepared lands which may cause to retain a smaller number of spores.

As per previous studies of Ilangamudali and Senarathnel (2016) and St. John (1980), root geometry with its age was found to be a consideration for the VAM and weed association. Plants with coarse root systems (Baylis, 1975) were more dependent on symbiotic fungi. According to this study, *Ageratum* spp., *Axonopus* spp., and *Drymaria* spp., which have shallow fibrous roots and *Oxalis* spp. which has woody tap root, were reported under higher colonization percentages (considering barren land). Further, *Borreria* spp., *Erigeron* spp. (considering cultivated land), *Drymaria* spp. and *Ageratum* spp. (considering only barren land) which have shallow fibrous roots were reported under higher spore counts. Thus, there must be a high possibility of having a relationship between higher spore population/root colonization percentages with the morphology of mycorrhizal weed root.

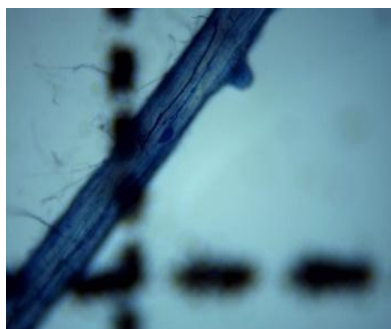


Figure 3: Observations of root colonies of mycorrhiza under compound microscope: root intersection of *Borreria* spp. from barren land under x400 magnification.



Figure 4: Observations of root colonies of mycorrhiza under compound microscope: *Borreria* spp. under x400 magnification from barren land.

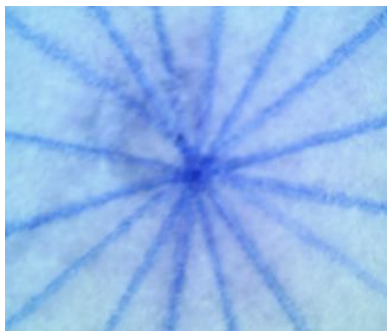


Figure 5: Observations of rhizosphere spores of mycorrhiza under dissecting microscopes: Whatman® filter paper under $\times 0.8$ magnification.

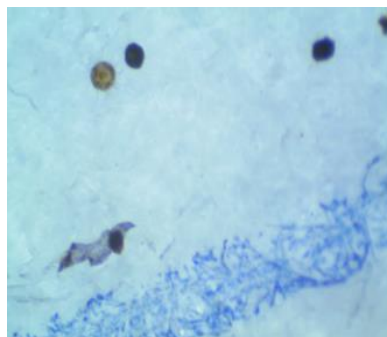


Figure 6: Observations of rhizosphere spores of mycorrhiza under dissecting microscopes: *Ageratum* spp. from cultivated land under $\times 5$ magnification.

The highest colonization percentage has recorded with *Axonopus* spp. (60.4%) whereas the lowest colonization percentage has recorded with *Eleusine* spp. (35.42%) compared to the *Erigeron* spp. (37.79%). The root colonization per cents were similar among all other weeds. The highest spore count has recorded with *Borreria* spp. (187 per 5 g soil). The lowest spore count has recorded with

Eleusine spp. (66 per 5 g soil) compared to *Axonopus* spp. (73 per 5 g soil). The spore populations were similar among *Bidens* spp. and *Oxalis* spp. only (Table 1).

Table 1: Variation in mean colonization percentage and spore count among weed species.

Weed species	Colonization percentage (%)	Spore count (per 5 g spoil sample)
<i>Ageratum</i> spp.	51.87 ^{ab}	157 ^a
<i>Axonopus</i> spp.	60.39 ^a	73 ^b
<i>Bidens</i> spp.	47.34 ^{ab}	111 ^{ab}
<i>Borreria</i> spp.	48.53 ^{ab}	187 ^a
<i>Cleome</i> spp.	50.21 ^{ab}	153 ^a
<i>Drymaria</i> spp.	52.22 ^{ab}	160 ^a
<i>Eleusine</i> spp.	35.42 ^{ab}	66 ^b
<i>Erigeron</i> spp.	37.79 ^b	161 ^a
<i>Oxalis</i> spp.	51.03 ^{ab}	111 ^{ab}

Note: Means that not share the same letters are significantly difference at $\alpha = 0.05$

Studies of Mafaziyal and Madawala (2015) recorded *A. compresses* with the highest colonization percentage (20%). Similarly, this study also reported the highest colonization percentage with *Axonopus* spp. According to the previous studies, *A. conyzoides* was reported as a VAM associated weed. As per those studies, 2% as the lowest (Mafaziyal and Madawala, 2015), 72.7% (Chawla *et al.*, 2011) as the highest and $54.46 \pm 6.18\%$ (Muthukumar and Prakash, 2009), 67% (tea land) (Hazarika, 2000) under medium level categories were recorded. Spore counts were recorded as 503.3 per 50 mL (Chawla *et al.*, 2011) under higher level category and 49.5 per 100 g soil (Hazarika, 2000) in a tea land under the lower-level category.

According to both colonization per cent (Figure 3 and Figure 4) and spore counts (Figure 5 and Figure 6) in the present study, *Ageratum* spp. can be placed under the higher VAM associated category. Muthukumar and Prakash (2009) recorded *Bidens* spp. under the medium level of $43.31 \pm 3.97\%$; similarly in the present case, it has recorded under medium level with 47.34%. According to the previous studies, weeds seemed to maintain the VAM population during the off-season (winter). During the spring and summer they may serve as a reservoir of inoculum of the natural mycorrhizal communities to other plants, influencing companion crops, trees, and health of the ecosystem. In winter season of tropical North India had supported low microbial activity (Chawla *et al.*, 2011), that proposed by St. John (1980) with the highest colonization percentage. Further, it is now widely accepted that climatic and edaphic factors (Ilangamudali and Senarathnel, 2016) and seasonal growth of their host spores (Abbott and Robson, 1991) can influence directly or indirectly by lesser numbers of VAM fungi spores. This is supported to figure out that, estimated

less mean root colonization percentages (<70%) and mean spore counts (<200 per 5 g) in this study may have caused due to the study period of North-East monsoon. As well as the age of weeds also can be a suspect for this reason. According to the findings of Chawla *et al.* (2011) *S. media* (family Caryophyllaceae) was reported under non-mycorrhizal.

However, *S. media* supported a good number of spores (350.0/50 mL of soil) in its root zone that had very shallow roots coupled with the presence of many mycorrhizal weeds (*Medicago* sp. and *Oxalis* spp.) in its area. It suspected that a large spore count of *S. media* in the soil may be due to surrounded roots host/non-host succession under field conditions that leftover resting structures like *Chlamydozoospores*. This suspicion also supported to make out that, *Erigeron* spp. of this study has not been recorded a large colonization percentage but a large spore count. According to the results of availability of soil P in VAM associated weeds, P levels in different 3 estates has not been significantly affected. Whereas difference in soil P level, between cultivated and barren land was significant ($P<0.01$). Further, P levels in different weed species were significantly affected ($P<0.01$). Mean soil P level of Wewesse, Spring-Valley and Telbedde estates have been reported as 6.0, 6.0 and 6.02 ppm, respectively, and there was no significant difference between any pair of estates hence soil P levels in all estates were more or less same.

Contradictory results on mean soil P level was significantly higher (6.12 ppm) in cultivated land than that of barren land (5.89 ppm) ($P<0.01$). Further, soil P level in all weeds except *Ageratum* spp. were resulted a higher P level in barren land than cultivated land (Figure 7). This results may be attributed to the regular application of tea fertilizer mixture on cultivated tea lands too.

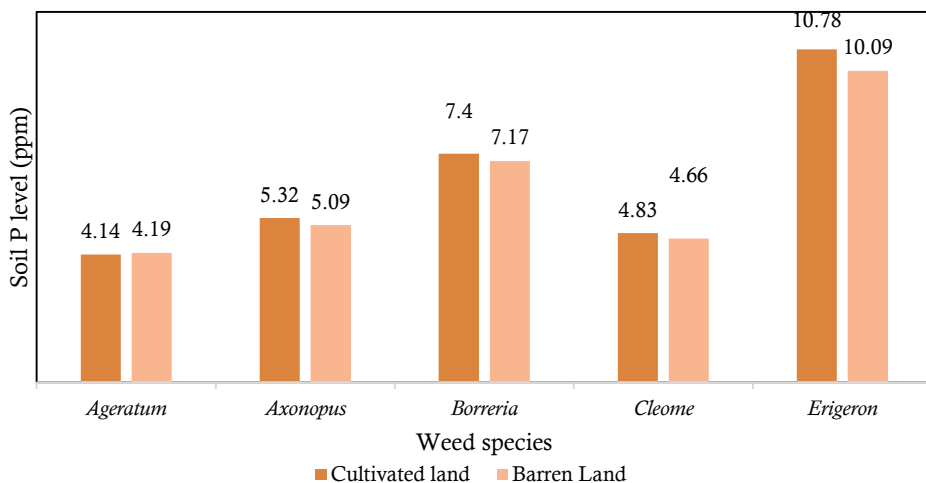


Figure 7: Mean soil P level reported in weed species under two land types.

However, considering only as weed species results for the P levels are reported as follows. The lowest soil P level was recorded in *Ageratum* spp. (4.17 ppm), which had the third highest colonization percentage and spore count. Soil P levels of *Cleome* spp. (4.74 ppm) and *Axonopus* spp. (5.20 ppm) were lower, where only the colonization percentages were higher. However, spore count in *Cleome* spp. was moderate but in *Axonopus* spp. spore count was recorded as the second lowest. Significantly the highest soil P level was quantified in *Erigeron* spp. (10.43 ppm), which was followed by *Borreria* spp. (7.28 ppm), where only the colonization percentage was recorded as lower.

Mycorrhizae benefit their host plants mainly by improving P uptake (Barea *et al.*, 1991; Clark and Zeto, 2000; Ward *et al.*, 2001; Javaid, 2007). Further, VAM secretes some phosphatases (Tarafdar and Marschner, 1994) and organic acids (i.e., oxalic acid) in the rhizosphere and catalyse them as P (Plenchette, 2005) and places at the disposal of the plants. This can be observed by the high P content of leaves (physiological expression) or less P content of the soil. These statements further supported to the present study as *Ageratum* spp. which recorded a higher colonization percentage and spore count recorded the lowest soil P level while *Erigeron* spp. which recorded a lower colonization percentage and spore count recorded as the highest soil P level. Thus, this study has supported to the fact that, both spore population and colonization percentage are negatively affected to the soil P level.

As per the results of impact on soil pH on VAM colonization and spore count in cultivated land mean soil pH at 19 ± 1 °C was 5.38. However, the soil pH was ranged from 4.22-6.20 and mode was 4.22. In barren land, mean soil pH at 19 ± 1 °C was 6.21. However, the soil pH ranged from 5.63 to 7.30 and mode was 5.63. Thus, soil pH level of the cultivated land can be ranged under (4.50 to 5.50) a standard tea cultivated land and barren land can be ranged under (6.00 to 7.00) neutral pH levels. As per Pallant's (2005) interpretations, this study also recorded a systematic moderate positive correlation between soil pH and root colonization percentage (Figure 8) at $P < 0.01$. Higher number of quantifications of colonization percentage were approximately recorded at 5.7 to 6.2 pH levels. Nevertheless, there was no systematic correlation between soil pH and spore count. However, higher number of quantifications of spore count were approximately recorded at 5.5 to 6 pH level (Figure 9).

Soil pH may affect the development and functioning of VAM, due to the alteration of the concentrations of nutrients, toxic ions and hydrogen ions in the soil solution (Hayman and Tavares, 1985). Also, the response of VAM to soil pH may depend on the species and strains belong to the indigenous VAM flora (Abbott and Robson, 1991). According to Aarle *et al.* (2002) soil pH around 6 positively influences the root colonization of extra radical mycelium of two mycorrhizal species (*Scutellospora calospora* and *Glomus intraradices*). As per study of Chawla *et al.* (2011) the soil pH of 6.3 at Pearson Road supported the higher root infection (70.0%). These findings have further supported to explain the

systematic positive relationship of colonization percentages with the increasing pH levels and higher number of quantifications of colonization percentage which are approximately around pH level of 6.00.

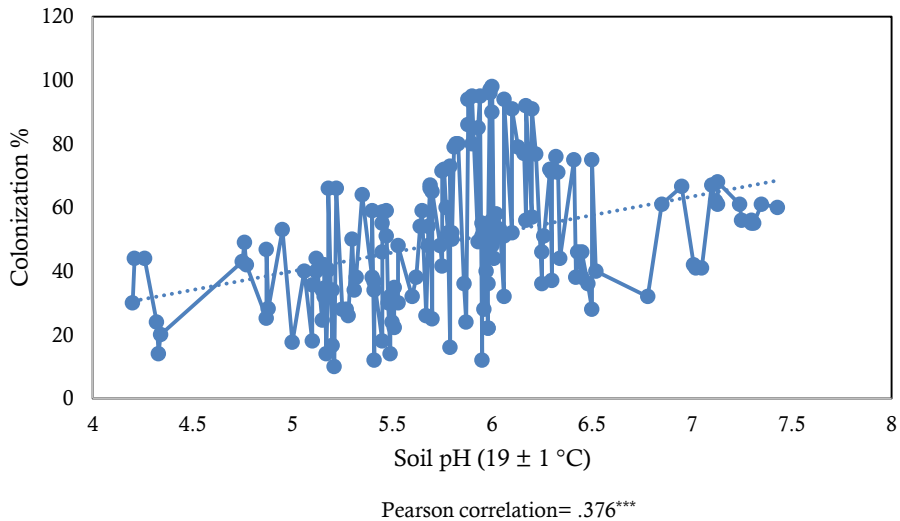


Figure 8: Variation of colonization percentages with soil pH levels (****P* values of statistics values<0.01, ***P* values of statistics values<0.05 and **P* values of statistics values<0.10).

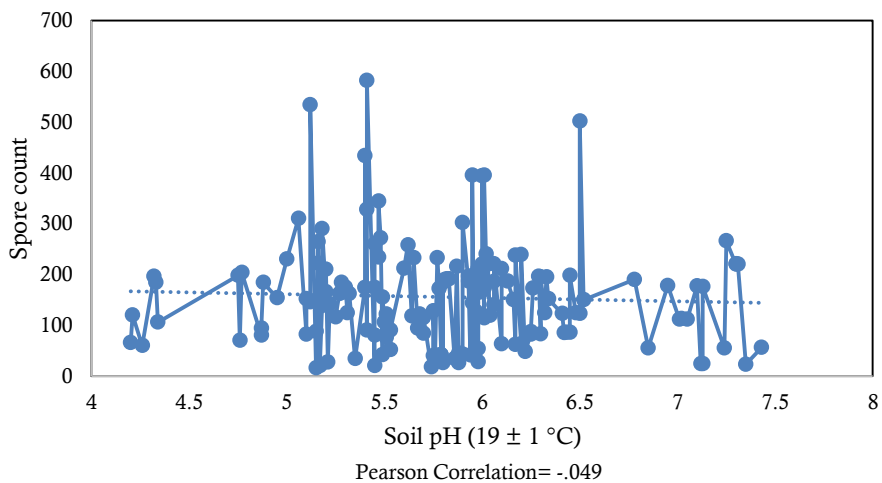


Figure 9: Variation of spore counts with soil pH levels (****P* values of statistics values<0.01, ***P* values of statistics values<0.05 and **P* values of statistics values<0.10).

CONCLUSIONS

There is a close association of VAM with all selected nine weed species. Weeds such as *Ageratum conyzoides*, *Axonopus compressus*, *Borreria latifolia*, *Cleome rutidosperma* and *Erigeron sumatrensis* were more prominent in VAM association among the selected weed species. The root colonization percentage and spore counts were varied significantly between different tea estates from the highest to medium level. In barren lands colonization percentage was significantly higher compared to the cultivated lands. Against this total mean spore count of barren lands was lower than that of cultivated lands. It was reported that soil P level is significantly higher in cultivated lands than in barren lands. The lowest and the highest soil P levels were found in *Ageratum* spp. and *Erigeron* spp., respectively, where a higher P level was also reported with *Axonopus* spp. In cultivated and barren lands soil pH was within the range of acidic and neutral, respectively. There is a systematic moderate positive correlation between soil pH and root colonization percentages but not between soil pH and spore counts.

Additionally, due to the lack of time and financial resources the coverage was limited only to the three estates of Badulla area which is in IM_{1a} agro-ecological zone of Uva region. However, it can follow the same research for some other weeds. Similarly, this study can be performed on further studying of other beneficial effects of such association between VAM and selected weeds. Besides, it can compare the VAM association, between VAM associated weeds and cover crops or green manure etc.

ACKNOWLEDGEMENT

Authors wish to acknowledge, Estate managers and staff of Wewesse, Spring-Valley and Telbedde estates, Badulla; Mr. P. Dias and Ms. L.N. Aluthge, for assisting in completing the statistical analysis of the study; Faculty of Animal Science and Export Agriculture and Faculty of Applied Sciences of Uva Wellassa University for providing with all the laboratory facilities. Parents, grand- parents, sisters, relatives and friends.

REFERENCES

- Aarle, I. M.V., Olsson, P.A. and Soderstrom, B. (2002) Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. *New Phytol.* 155, 173-182.
- Abbott, L.K., Robson, A.D. (1991). Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture, Ecosystems and Environment.* 35, 121-150.
- Azcon, R., Gomes, M., Tobart, R. (1996). Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought stress conditions. *Biol. Fert. Soils* 22, 156-161.
- Balasuriya, A., Arulpragasam P.V. and Ratnayake, R.M.A. (1991). Mycorrhiza in tea. *Tea Bull.* 11 (1/2), 03-12.

- Barea, J.M., Azcón-Aguilar, C., Azcón, R. (1991). The role of VA mycorrhizae in improving plant N acquisition from soil as assessed with ^{15}N . In: Stable isotopes in plant nutrition. IAEA, Vienna, pp 209–216.
- Barthakur, N.N. and Arnold, N.P. (1989). Certain organic and inorganic constituents in ba el (aegle marmelos correa) fruit. *Trop. Agric.* 66, 65-8.
- Baylis, G.T.S. (1975). The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders, F.E., Mosse, B., Tinker, P.B. (Eds), *Endomycorrhizas*. Academic Press, New York. pp. 373-389.
- Blatchley, W.S. (1912). *The indiana weed book*. Nature Publishing Company.
- Breuillin, F., Schramm, J., Hajirezaei, M., Ahkami, A., Favre, P., Druege, U., Hause, B., Bucher, M., Kretzschmar, T., Bossolini, E., *et al.*, (2010). Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. *Plant, J.* 64, 1002–1017.
- Brundrett, M. (2008). Mycorrhizal associations: The web resource, version 2.0. (online) (Accessed on 22.10.2018) Available at <https://mycorrhizas.info/info.html>.
- Brundrett, M.C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil.* 320, 37-77.
- Chawla, A., Singh, Y.P., Singh, P., Rawat, S., Das, S. and Singh, N. (2011). Ecological significance of mycotrohy in some tropical weeds. *Tropical Ecology.* 52(3), 303-310.
- Clark, R.B. and Zeto, S.K. (2000). Mineral acquisition by arbuscular mycirrhizal plants. *Journal of Plant Nutrients.* 23, 867-902.
- Daniel, B.A. and Skipper, H.D. (1982). Method for the recovery and quantitative estimation of the propagule from soil. pp. 26-29.
- De Silva, M.S.D.L. (2016). *Integrated weed management in tea lands; alternatives for glyphosate*. Tea Research Institute of Sri Lanka.
- De Silva, M.S.D.L., Rajasinghe, J.C.K. and Mahindapala, K.G.J.P. (2003). Weeds of tea land in sri lanka. Tea Research Institute of Sri Lanka.
- George, E., Gorgus, E., Schmeisser, A., Marschner, H. (1996). A method to measure nutrient uptake from soil by mycorrhizal hyphae. In: *Mycorrhizas in integrated system from genes to plant development* (Eds). Luxembourg. European Community.
- George, E., Romheld, V., Marschner, H. (1994). Contribution of mycorrhizal fungi to micronutrient uptake by plants. In: Monthey, J.A., Crowley, D.E. and Luster, D.G. (Eds), *Biochemistry of metal micronutrients in the rhizosphere*. Boca Raton FL CRC Press. pp. 93-109.
- Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society.* 46, 235-244.
- Giovanneti, M. and Mosse, B. (1980). An evaluation of technique for measuring arbuscular mycorrhizal infection in the roots. *New Phytologist.* 84, 489-500.

- Guo, Y., George, E., Marschner, H. (1996). Contribution of an arbuscular mycorrhizal fungus to the uptake of cadmium and nickel in bean by maize plants. *Plant Soil*. 184, 195-205.
- Hayman, D.S. and Tavares, M. (1985). Plant growth response to vesicular arbuscular mycorrhiza. Influence of soil pH on the symbiotic efficiency of different endophytes. *New Phytologist*. 100, 367-378.
- Hazarika, D.K. (2000). Occurrence and significance of vesicular arbuscular mycorrhizal (Vam) Fungi in Tea Garden Soils of Assam. Assam Agricultural University. 97-A (D)-10.
- Ilangamudalil, I.M.P.S. and Senarathnel, S.H.S. (2016). Effectiveness of arbuscular mycorrhizal fungi-based bio fertilizer on early growth of coconut seedlings. *Cocos*. 22, 01-12.
- Jacobsen, I., Abbott, L.K. and Robson, A. (1992). External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trofoluim subterraneum* L. I. Spread of hyphae and phosphorus inflow into roots. *New Phytol*. 120, 371-380.
- Javaid, A. (2007). Allelopathic interactions in mycorrhizal associations. *Allelopathy Journal*. 20, 29-42.
- Jeffries, P. (1987). Use of mycorrhiza in agriculture. *Crit. Rev. Biotechnol*. 5, 319-357.
- Jordan, N.R., Zhang, J., Huerd, S. (2000). Arbuscular-mycorrhizal fungi: potential roles in weed management. *Weed Research*.40, 397-410.
- Lambert, D.H., Baker, D.E., Cole, H.J. (1979). The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. *Soil Sci. Soc. Am. J.* 43, 976-980.
- Liu, A., Hamel, C., Elmi, A., Costa, C., Ma, B., Smith, D.L. (2002). Concentrations of K, Ca and Mg in maize colonised by arbuscular mycorrhizal fungi under field conditions. *Can. J. Soil Sci.* 82(3): 271278.
- Lk. geoview.info. (2021). *Province of Uva*. [online] Available at: <<https://lk.geoview.info/uva>> [Accessed 3 April 2021].
- Mafaziyal, F. and Madawala, S. (2015). Abundance, richness and root colonization of arbuscular mycorrhizal fungi in natural and semi-natural land use types at upper hantana. *Ceylon Journal of Science (Bio. Sci.)* 44 (1).
- Muthukumar, T. and Prakash, S. (2009). Arbuscular mycorrhizal morphology in crops and associated weeds in tropical agro-ecosystems. *Mycoscience*. 50, 233–239.
- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1988). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture Circle 939.
- Olsson, P.A., Rahm, J. and Aliasghar zad, N. (2010). Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiol. Ecol*. 72, 123 -131.
- Ortas I., Harries P.J. and Rowell, D.I. (1996). Enhanced uptake of phosphorus by mycorrhizal sorghum plants as influenced by the form of nitrogen. *Plant Soil*. 184, 255-264.
- Pallant, J. (2005). SPSS survival manual: A step by step guide to data analysis using SPSS for windows (version 12). Second ed. National Library of Australia.

- Phillips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*. 55, 158-161.
- Plenchette, C. (2005). Mycorhizes et nutriments phosphate des plantes. *journees techniques fruits et legumes*. Viticulture Biologiques. 103-109.
- Quilambo, O.A. (2003). The vesicular-arbuscular mycorrhizal symbiosis. *African Journal of Biotechnology*. Eduardo Mondlane University. 2 (12), 539-546.
- Rathi, S.K. and Singh, L. (1990). Preliminary survey of mycorrhizal fungi in some weeds and cultivated plants in Meerut. *Proc. Workshop on Mycorrhizae*. Haryana Agricultural University, Hissar. pp. 31- 32.
- Road Development Authority, Ministry of Higher Education and Highways for the Government of Sri Lanka and the Asian Development Bank. (2017). SRI: Second integrated road investment program upva province. Initial Environmental 6 Examination, Road Development Authority.
- Sieverding, E., (1991). Vesicular arbuscular mycorrhizal management in tropical agrosystems. *Gesellschaft für Technische Zusammenarbeit (GTZ)*. Eschborn, Germany.
- Singh, K. and Varma, A. K. (1981). Endogonaceous spores associated with xerophytic plants in northern India; *Trans. Br. Mycol. Soc.* 77, 655-658.
- Smith, S.E., Read, D.J. (1997). *Mycorrhizal symbiosis*. Academic Press, Inc. San Diego California. ISBN 012-652840-3.
- St. John, T.V. (1980). Uma lista de espécies de plantas tropicais brasileiras naturalmente infectadas com micorriza vesicular-arbuscular. *Acta Amazonica*. 10, 229-234.
- Subramanian, K.S and Charest, C. (1999). Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions. *Mycorrhiza*. 9, 69–75.
- Tarafdar, J.E., Marschner, H. (1994). Efficiency of VAM hyphae in utilization of organic phosphorus by wheat plants. *Soil Science, Plant Nutrition*. 40, 593-600.
- Verbruggen, E., and Kiers, E.T. (2010). Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. *Evol. Appl.* 3, 547–560.
- Viire, H., Vestberg, M. and EuroJa, S. (1992). Mycorrhiza and root-associated fungi in Spitsbergen. *Mycorrhiza*. 1, 93-104.
- Ward, R., Stead, K. and Reeves, J. (2001). Impact of endomycorrhizal fungi on plant trace element uptake and nutrient. *Nutrient Practice* 32, 30-31.
- Wijeratne, M.A. and Chandrapala, L. (2014). *Climatic variations in tea growing regions and vulnerability of tea plantations to climate change*. Tea Research Institute of Sri Lanka.