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MULBERRY JUICE AND SOAP POWDER, A SAMPLE STAINING FARMER METHOD FOR ARBUSCULAR-MYCORRHIZAL FUNGI IN INVESTIGATE OF SOIL HEALTH

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ABSTRACT

Soil health is the capacity of soil to function as a vital living system. Sustainable agriculture requires increasing farmer knowledge about the soil health that's could be by assess mycorrhizal fungi. This study investigated the development and simplified method of soil tests which farmers could use on their own farms. The tests would enable monitoring of mycorrhizal fungi on-farm. The goals of this research were to develop and test modified methods for monitoring mycorrhizas fungi that require minimal specialist equipment. Standard laboratory methods for staining mycorrhizal fungi in roots (Ink-vinegar and Trypan Blue methods) were adapted. It was possible to simplify the method so it was more suitable for farmers to use than the methods used in the laboratory. the method for clearing prior to staining roots for mycorrhizal fungi was simplified for farmers by replacing KOH with soap powder an evaluation of this simplification was made as was an evaluation of the use of commonly available organic dyes as stains (mulberry juice) instead of Ink or Trypan Blue for staining the mycorrhizal fungi.

INTRODUCTION

The farmers need to understand their soil health to sustain and manage their farming systems. They can monitor soil health by a simple farmer method to staining of Arbuscular - mycorrhizal fungi because it could be the best indicator of the soil health because mycorrhizal fungi have a significant role in contributing to improve the ecosystem (3). it has a role of transport and uptake of nutrients and water (2; 12).Mycorrhizal hyphae can help the transport of nutrients such as phosphorus and micronutrients into plants (5;7;13) and they also have a role in development and maintenance of soil structure. There are significant symbiotic relationships between mycorrhizal fungi and plants in soil especially in crop and pasture species (13). Therefore, they have potential to contribute to cropping system productivity and ecosystem sustainability (4; 6) Mycorrhizal fungi represent a major component of the microbial soil community (3). Their role in soil aggregation enables them to contribute to storing carbon in soil (5; 8; 11)

Roots were cleared and stained to show the presence of arbuscular mycorrhiza fungi using two common methods; KOH - HCl - Trypan Blue (9) and KOH - Ink – vinegar (16) methods. In both cases, the roots were covered with 10% potassium hydroxide (KOH) for 4-5 days to clear the cytoplasm in the root cells following that, the fungi was stained with either 5% ink-vinegar for a day or with 0.05% Trypan blue (16).

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In the both methods that using chemicals materials such as KOH and HCl. it is a dangerous on the farmers because may don't used it before. Therefore, the method for clearing prior to staining roots for mycorrhizal fungi was simplified for farmers by replacing KOH with soap powder because soap powder has Sodium hydroxide. An evaluation of this simplification was made as was an evaluation of the use of commonly available organic dyes as stains (mulberry juice) instead of Ink or Trypan Blue for staining the mycorrhizal fungi.

Low-technology method was developed because farmers would not have access to dangerous or expensive chemicals (16) and also because the staining of mycorrhizas was adequately clear. In addition, we developed new methods by using accessible materials such as soap powder to clearing the roots and using the black colour of mulberry juice for staining the roots. This could be studied further because, Mulberry juice and soap powder method was very stable and easy to use by farmers and does not use toxic chemicals and if preferable for on farm use. This method was suitable for staining all fungal structures, rendering them clearly visible.

MATERIALS AND METHODS

(i) Staining roots with the KOH- Ink – Vinegar Method

Ryegrass and clover roots were sampled and washed with tap water. They were cut into 1 cm pieces and a subsample of 0.1 g of roots (with 6 replicates for both plant species) was placed in small test tubes and covered with 10% potassium hydroxide (KOH) (9) for 4-5 days to clear cytoplasm in the cells (16) prior to staining the fungal hyphae with ink and vinegar for 12h, roots were rinsed several times with tap water. For a faster method, some roots were boiled for 5 min at 90°C before rinsing several times with tap water prior to addition of the 5% ink-vinegar and boiled for 1 min before pouring off the stain then destaining with either tap water or lacto glycerol. The proportion of roots that contained mycorrhizal fungi was determined under a dissecting microscope (20 x magnifications). Stained root samples were stored at room temperature for several weeks (16).

(ii) Staining roots with the KOH- Trypan Blue

As for staining with Ink described above, ryegrass and clover roots were sampled and washed with tap water for staining with Trypan Blue (9).

(iii) Clearing roots using KOH

1 gm roots of ryegrass or clover roots, gar, Hotplate, beaker, forceps, and small plastics tube with caps, 10% potassium hydroxide (KOH) (9).

Ryegrass and clover roots were washed with tap water, they were cut into 1 cm pieces and weight subsample of 0.1 gm of roots, placed in small test tubes and covered with 10% potassium hydroxide (KOH) for 4-5 days to clear cytoplasm in the cells, or to be faster can boil them for 5 mint at 90°C after that rinse (9) The time for clearing of root tissues must be adjusted depending upon the plant species studied (16).

(iv) Clearing roots using soap powder

It was considered important to develop an easy and simple method to assess mycorrhizal fungi because farmers are not familiar with using the chemicals that are normally used in laboratory settings (such as KOH) (9). In this study, I developed a new method to stain roots using the juice of mulberries

(*Morus* sp.) (14 of ink or Trypan blue stains. Roots were sampled as described in (i) above.

(v) Clearing and staining roots using soap powder and mulberry juice

The black juice of berries was used at full maturity in the ratio of 3:1 mulberry juice to white 5% vinegar. Ryegrass and clover roots were sampled and washed with tap water. They were cut into 1 cm pieces and a subsample of 0.1 gm of roots (with 6 replicates for both plant species) was placed in small test tubes and covered with soap powder (1gm/100 mLwater) for 4-6 days to remove cytoplasm in the cells The time for clearing of root tissues must be adjusted depending upon the plant species studied (16). Prior to staining the fungal hyphae with black juice of for 24 hrs. After that, the roots were rinsed several times with tap water. Mycorrhizas were assessed as above.

RESULT AND DISCUSSION

Two common methods (Ink- Vinegar, Trypan Blue) were assessed for arbuscular mycorrhizal fungi in plant root. They were good staining roots. However, Trypan Blue gave higher percentage of mycorrhiza colonisation than Ink and Vinegar method for both plants.

I developed two processing methods, one of them to clearing roots and another to staining roots by using accessible materials such as soap powder to clearing the roots and using the black colour of mulberry to staining the roots.

In addition, there were some roots which were cleared by powder soap were tested with the three kind of colours (Ink, Trypan Blue and black colour of Mulberries).

(i) Staining roots with KOH- Ink- Vinegar

Plant (ryegrass) roots stained using the Ink and Vinegar method were colonised (46 %) to a similar extent to the clover roots (45 %). In addition, colonized roots were easily distinguished from non-colonized roots. Also, individual hyphae in heavily and partially colonised sections of roots were clearly visible. Arbuscules, vesicles, intraradical and extra radical hyphae, and appressoria were all stained by this staining method (Figure 4).

(ii) Staining roots with KOH- Trypan Blue

Ryegrass roots stained with Trypan blue had a mean of root length colonised of 64 % similar to clover roots (65 %). Therefore, mycorrhizal colonization stained by the Trypan Blue method appeared to have a higher percentage of mycorrhiza colonization than the Ink and Vinegar method for both plants (Figure 1). For both methods, the colonised roots were easily distinguished from non-colonised roots. Also, individual hyphae in heavily and partially colonised sections of roots were clearly visible. Arbuscules, vesicles, intraradical and extra radical hyphae, and appressoria were all stained well by both methods, but for the Trypan blue method, the staining of the clover roots was darker than for ryegrass roots. However, the staining of ryegrass roots with Ink- vinegar method was darker than for the clover roots. Generally, the staining of roots by Trypan Blue was more effective than the staining by the Ink- vinegar method.

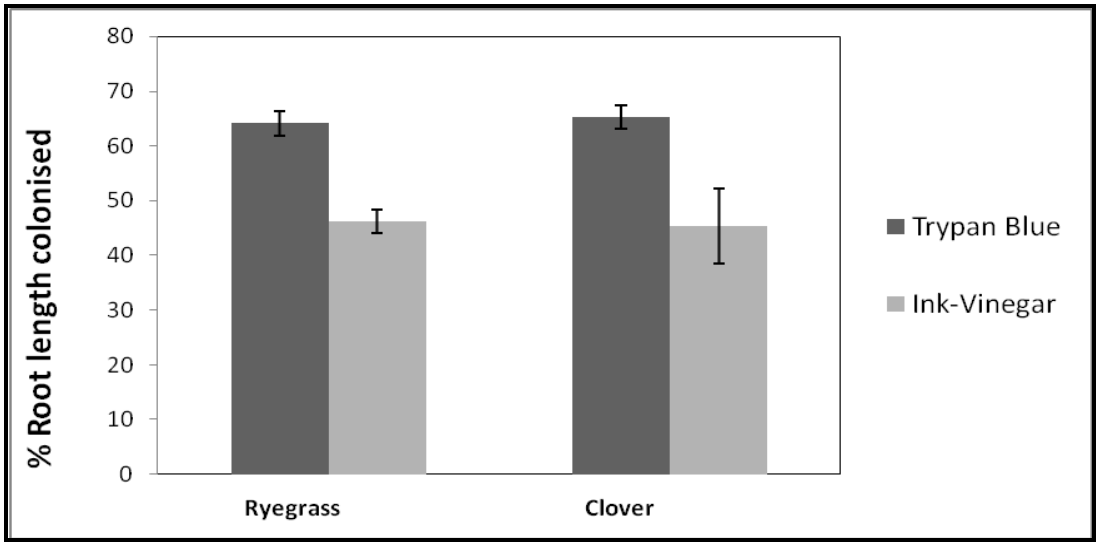

Fig. 1: Comparison between staining methods for arbuscular mycorrhizal fungi.

(iii Clearing and staining roots using soap powder and mulberry juice

This method was successful because the materials of this method are safe for use by farmers, therefore the low-technology methods were developed because farmers would not have access to dangerous or expensive chemicals. Therefore, decreasing chemicals is important reasons for safety because they could cause irritate the eyes nose, throat, lungs and skin irritation (1; 10, 15).

Clearing and staining roots using soap and staining of roots with black mulberry juice at Raygrass plant was more than Clover plant (Figure 2).

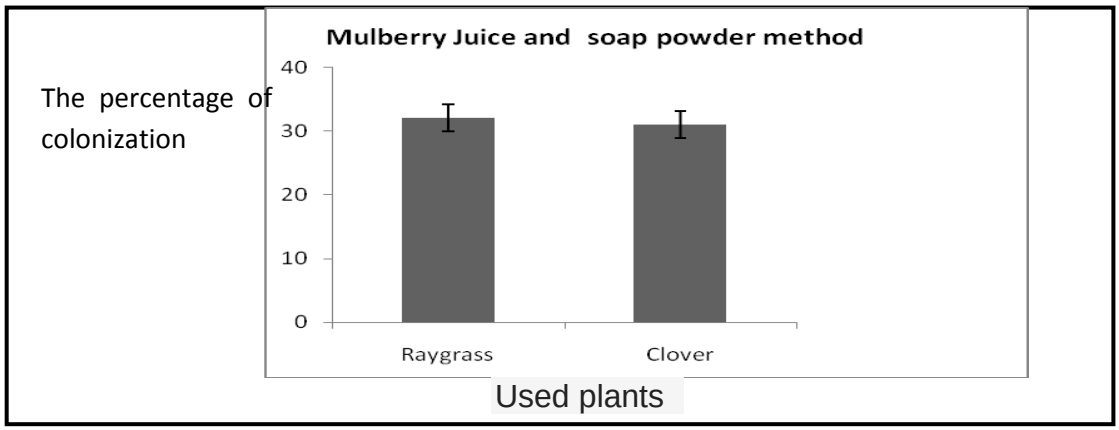

Fig. 2: Mycorrhizal staining with using black mulberry juice and soap powder.

Also, roots were good clearing with powder soap (Figure3), and the colonized roots were easily distinguished with using black mulberry juice, also individual hyphae in heavily and partially colonised sections of roots were clearly visible (Figure 4 and 5). Clearing and staining roots using soap and staining of roots with black mulberry juice was effective but not as clear as Trypan blue. Individual hyphae and partially colonised sections of a ryegrass roots were clearly visible when stained with black mulberry juice, as were arbuscules, vesicles, intra radical and extra radical hyphae and points of penetration (Figure4).

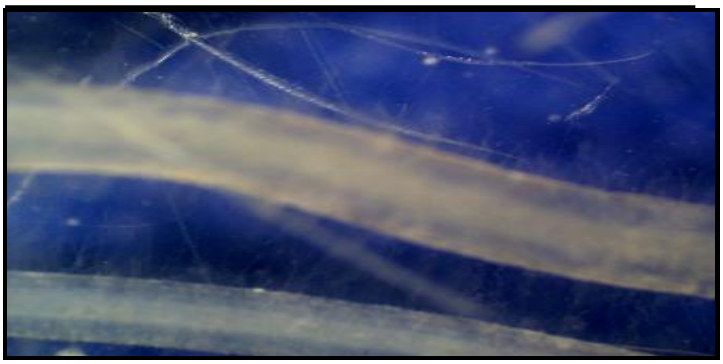

Fig. 3: clearing ryegrass roots by powder soap.

Although the clearing of roots by KOH (11) is high quality method and better than clearing using soap powder, it is safe and easy to use by farmers. The result of clearing roots by soap was satisfaction but not as good as when KOH was used (Figure 1). When the roots were cleared using soap powder with two stains (Ink, Trypan Blue) the following was observed:

(i) Clearing and staining roots using the soap and ink-vinegar method showed that after an adequate clearing time, good staining of mycorhizas occurred, especially external and internal hyphae. Colonised roots were easily distinguished (Figure 6).

(ii) Clearing and staining roots using soap and Trypan Blue was very good effective for staining arbuscules and internal hyphae in ryegrass and clover roots (Figure 7).

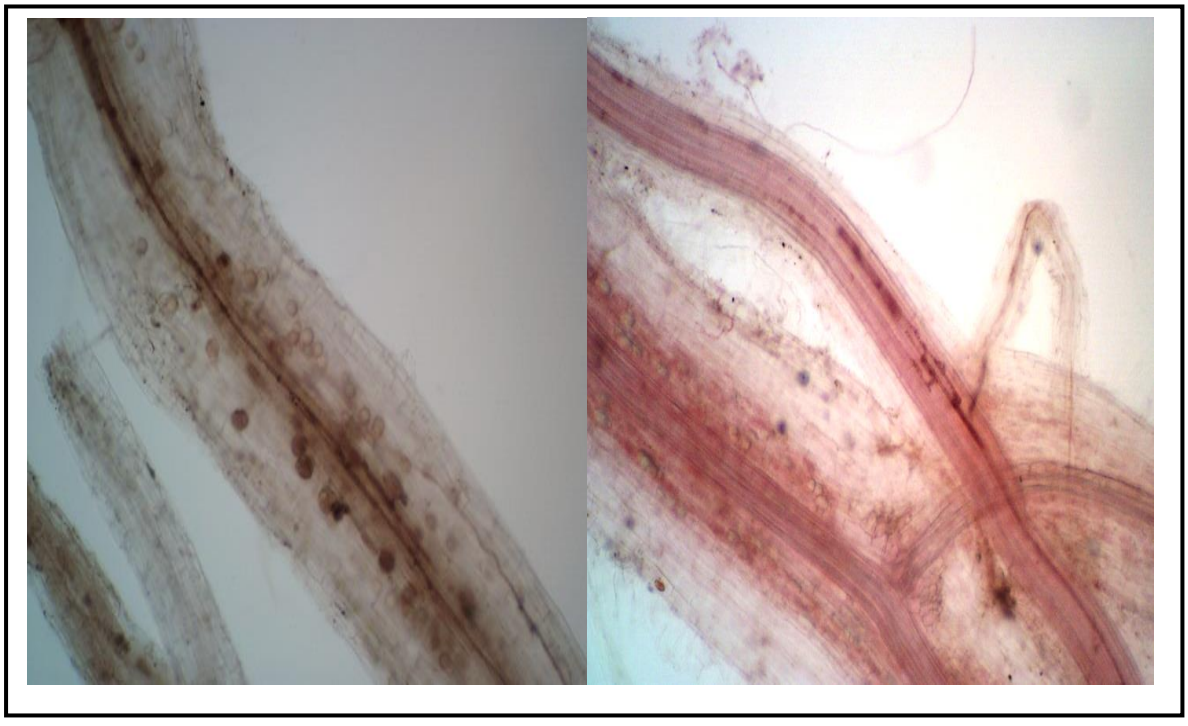

Fig.4: clearing and staining root by powder soap and black colour of Mulberries. (Arbuscules in ryegrass root tissue).

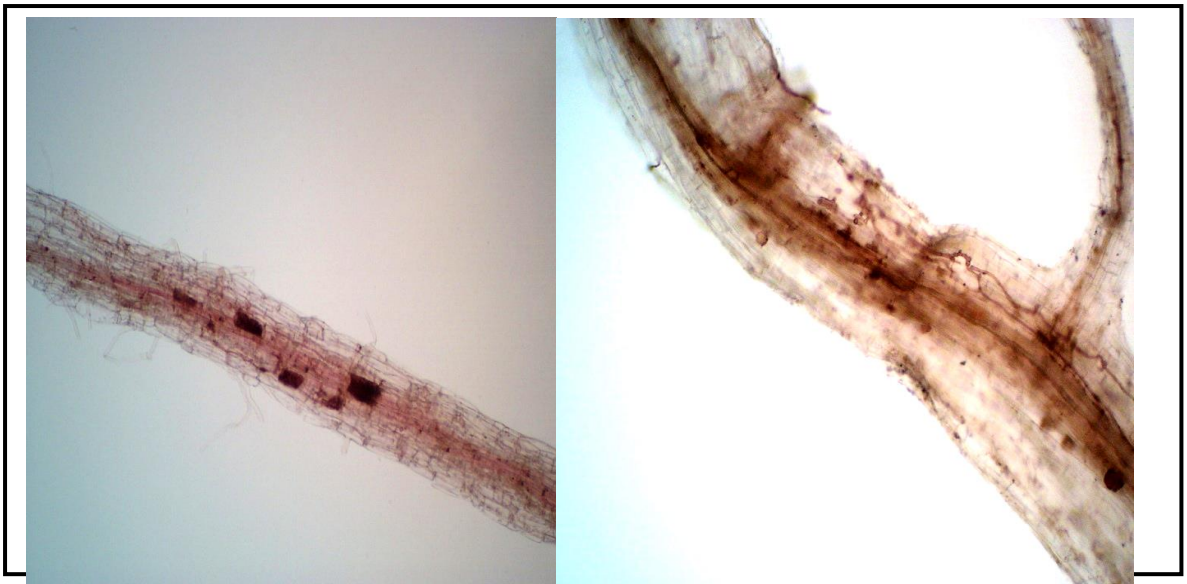

Fig. 5: Arbuscules and internal hyphae in ryegrass root tissue.

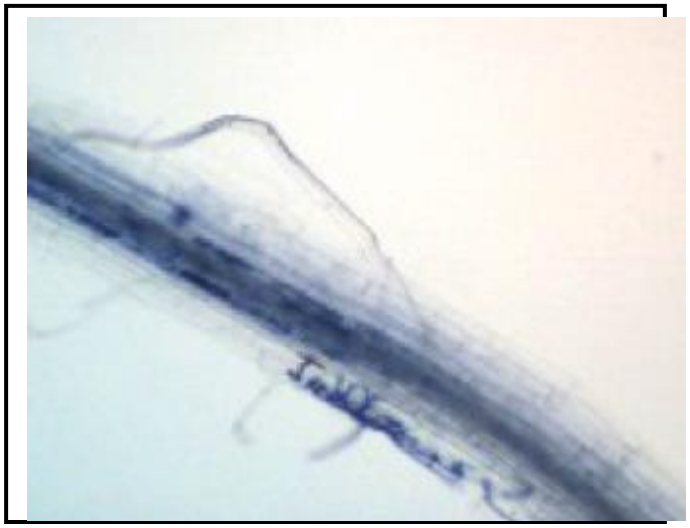

Fig. 6: clearing and staining ryegrass root by powder Soap and Ink- vinegar (Arbuscules, external and Internal hyphae in ryegrass root tissue).

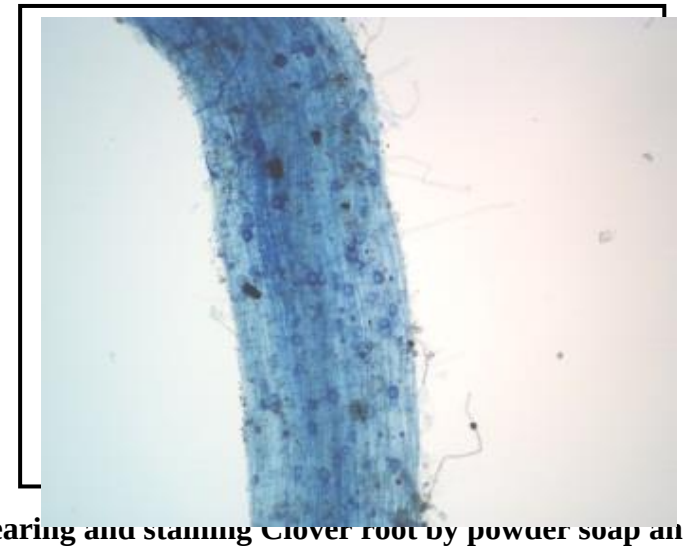

Fig. 7: clearing and staining Clover root by powder soap and Trypan Blue.

CONCLUSION

The simplify farmer method for staining Aarbuscular- mycorrhizal fungal colonizations in root tissues which is more suitable for farmers to use than the methods used in the laboratory. This method is a reliable, economical, nontoxic and easy for staining Aarbuscular - mycorrhizal fungal colonizations also could be of great utility in teaching exercises because as was an evaluation of the use of commonly available organic dyes such as replacing KOH with soap powder and mulberry juice instead of Ink or Trypan Blue for staining the mycorrhizal fungi on their own farms in order investigate of soil health.

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استخدام عصير التوت وصابون الغسيل لتشخيص الفطريات الجذرية (المايكورازيا) وذلك للتحقق من صحة التربة

جواد عناد مهدي * لين ابوت** زكريا سليمان**

الملخص

صحة التربة هي قدرة التربة لتكون بمثابة نظام معيشي حيوي، تتطلب الزراعة المستديمة زيادة معرفة الفلاح بشأن صحة التربة وذلك عن طريق حساب وقياس فطريات المايكورازيا في التربة.

تهدف الدراسة الى تطوير طريقة مبسطة لاختبار التربة بواسطة المزارع نفسه في مزرعته الخاصة.

يتطلب حساب فطريات المايكورازيا الى استخدام مواد مختبرية خاصة وفي الطريقة المختبرية لتصبيغ الجذور هو استخدام مواد مختبرية مثل:

– صبغة الحبر والخل.
– صبغة ترايبن بلو.
– هيدروكسيد البوتاسيوم.

وكان من الممكن تبسيط هذه المواد لاستخدام مواد بسيطة بدل من المواد المختبرية. ومن هذه المواد البسيطة استخدام صبغة التوت الاسود بدلاً من صبغة الحبر والخل او صبغة ترايبن بلو لتصبيغ فطريات المايكورازيا، وكذلك استخدام مسحوق الصابون بدلاً من هيدروكسيد البوتاسيوم لزيادة شفافية الجذور.

وقد توصلت الدراسة الى جملة نتائج وكما يأتي:

– تبين ان شفافية الجذور كانت جيدة باستخدام مسحوق الصابون.
– التصبيغ بواسطة صبغة التوت الاسود كانت جيدة بالمقارنة مع استخدام صبغتي الحبر او ترابين بلو.

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