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Vegetable Seed Germination Enhancement Using Different Levels of Pyroligneous Acid (PA)

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Abstract
Different concentration of pyroligneous acid in priming solutions were tested on the germination of four (4) different vegetable seeds such as radish, tomato, cucumber and eggplant. Priming solutions were consist of 5%, 10%, 15% and 20% pyroligneous acid used to prime the seeds of eggplant and tomato for twenty four (24) hours while sixteen (16) hours for cucumber and radish seeds. Germination test were done in dark room condition using petri dishes containing 50 seeds each with three replications. Fastest rate of germination were observed on radish and cucumber seeds with only two days reaching 100 % germination. Final germination percentage of tomato and eggplant seeds treated with pyroligneous acid increased with 7% and 4 % difference compared to untreated seeds. Priming enhances the germination of tested vegetable seeds ranging from 93 to 100% germination in all treatments of all seeds except for 20% PA (T5) in eggplant seeds with 76% FGP. Studies dealing with the different number of days of seed priming, application of PA during seedling emergence, vegetative, and reproductive stage of plants is suggested to fully explore its potential as organic fertilizer and growth enhancer.
Keywords: pyroligneous acid, priming, vegetable seeds, speed of germination, initial germination percentage, final germination percentage.

1. INTRODUCTION

Pyrolysis is the thermal decomposition of organic material in the absence of oxygen or when the oxygen is present in amount significantly less than required for complete combustion. Wood pyrolysis is a route for the production of charcoal (biochar-solid), tar (liquid) and gaseous products as possible alternate sources of energy. Pyroligneous acid (PA) or wood vinegar is a dark brown solution obtained as a by-product of wood carbonization. It contains over 200 components, such as acids, alcohols, phenols and neutrals (Jodai et al., 1989; Shirakawa et al., 1995b; Yatagai et al., 1998). PA accelerates seed germination of garland chrysanthemum (Chrysanthemum coronarium L.) honewort (Cryptotaena sativa L.) and lettuce (Lactuca sativa L.)(Uehara et al,. 1993), seed germination and plantlet growth of Chinese cabbage (Brassica campestris L.) and rice (Oryzza sativa L.) (Yatagai and Unrinin, 1987), (Shirakawa et al., 1995a) growth of rice seedlings, and root formation of sweet potato (Ipomoea batatas Poir.) plantlets (Du et al., 1998).

Recently, there have been growing interests in the analysis of chemical constituents of pyroligneous acid. Large number of substances has been found in the pyrolysis liquids from different resources. The detected substances from the acids belong to different classes of organic compounds, namely, aldehydes, ketones, alcohols, organic acids, esters, derivatives of furan and pyran, phenolic and neutral, hydrocarbons and nitrogen compounds, in which the major ones are organic acids and phenolic. (Zulkarami et al., 2011), used Inductively Coupled Plasma Mass-Spectrometry (ICP-MS). Analysis of the pyroligneous acid samples revealed the presence of 15 elements. The elements detected were calcium, cadmium, chromium, copper, iron, potassium, magnesium, lead, aluminum, sodium, zinc, arsenic, boron, molybdenum and phosphorus. Highest concentrations were recorded for calcium, iron and sodium with levels of 8.82, 5.80 and 3.93 ppm, respectively.

Cadmium, copper, lead, arsenic, zinc, boron, molybdenum and phosphorus were present at very low levels, while chromium, potassium, magnesium and aluminum were present at intermediate levels. Semi-volatile organic compounds detected in pyroligneous acid were pyrogallol 1,3-dimethyl ether, 2-methoxy-benzeneethanol, 1,2,3-trimethoxy-5-methyl benzene, 3-(o-azidophenyl) propanol, N-(dimethylthiophosphinyl)-3-amino pyridine, 2-pyridinepropanoic acid and 2,5-dimethylphenol. Compounds present in significant quantities were pyrogallol 1,3-dimethyl ether (93 %), followed by 2-methoxy-benzeneethanol (90 %), and 1,2,3-trimethoxy-5-methylbenzene (80 %). The pyroligneous acid comprised of several major volatile compounds such as pyrogallol 1,3-dimethyl ether, 2-methoxy-benzeneethanol and 1,2,3-trimethoxy-5-methyl benzene, which accounts for the final aroma (Natera et al., 2002). The aroma component determines the quality of the pyroligneous acid. These include alcohols, esters, carbonyls, acids, phenols, lactones and acetals (Callejón et al., 2008). Even though the chemical characteristics of the main active compounds in pyroligneous acid has only in recent times been discovered (Van Staden et al., 2004), the remarkable effect of smoke on seed germination is widely known and utilized in various ways (Roche et al., 1997: Brown and Van Staden, 1997; Van Staden et al., 2000).

Heat is not required for the germination response, as cold smoke application induced an up to 48-fold increase in the number of germinating seedlings and approximately 3-fold enrichment in species abundance in field trials (Roche et al., 1997;Rokich et al., 2002). It has now been well established that smoke is a broadly effective stimulant that enhances germination of approximately 1,200 species in more than 80 genera worldwide (Dixon et al., 2009). Attempts to study smoke effects on plant physiology have been confounded by the complex mixture of components within smoke, some of which confer toxicity at high concentrations. Bioassay-guided fractionation of smoke water culminated in the discovery and synthesis of the primary germination stimulant 3-methyl-2*H*-furo [2,3-*c*]pyran-2-one (KAR_1; Flematti et al., 2004). With the recent identification of three analogous active compounds in smoke water fractions (Fig. 1A; G. Flematti, unpublished data), this family of butenolide molecules have been designated karrikins, after "karrik," the first recorded Aboriginal Nyungar word for smoke (Dixon et al., 2009). Partial structural similarity between the karrikin family of plant growth regulators and strigolactones. A molecular structures of KAR_1 (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one), KAR_2 (2*H*-furo[2,3-*c*]pyran-2-one), KAR_3 (3,5-dimethyl-2*H*-furo[2,3-*c*]pyran-2-one),**...** The parent molecule, KAR_1, is a potent stimulant that enhances germination in some species at sub-Nano molar concentrations (Flematti et al., 2004; Stevens et al., 2007). In field trials, KAR_1 is effective at less than 5 g ha^{-1} compared with 10 ton ha smoke water and thus may have practical value in agriculture, conservation, and restoration (Stevens et al., 2007).

Smoke water fractions containing KAR_1 have been reported to enhance seedling vigor of several weed and crop species, indicating potential use for KAR_1 as a seed priming agent to improve germination and seedling establishment (Jain et al., 2006; Jain and Van Staden, 2006; Kulkarni et al., 2006; van Staden et al., 2006; Daws et al., 2007a). Since its discovery, a widespread capacity for KAR_1 germination response among angiosperms has been demonstrated (Flematti et al., 2004; van Staden et al., 2004, 2006;Merritt et al., 2006; Daws et al., 2007a; Stevens et al., 2007). Thus, karrikins may be considered a novel class of plant growth regulators with broad impact.

This study was conducted to find out the effect of different levels of pyroligneous acid in priming solution in relation to seed germination of four different vegetable seed cultivars in terms of the initial germination percentage, speed of germination and final germination percentage.

1.1 MATERIALS & METHODS

1.1.1 Pre-priming activities

Vegetable seeds were disinfected with 1% Sodium Hypochlorite solution for 15 minutes to eliminate seed-borne microorganisms. Following disinfestation, seeds were rinsed in running tap water for one (1) minute and surface dry by placing them between tissue papers for thirty (30) minutes under room temperature.

Solutions were prepared by dissolving pyroligneous acid and distilled water using the following treatments:

T1-0% pyroligneous acid + 100% (distilled water)
T2-5% pyroligneous acid + 95% distilled water
T3- 10% pyroligneous acid + 90% distilled water
T4- 15% pyroligneous acid + 85% distilled water
T5- 20% pyroligneous acid + 80% distilled water

1.1.2 Priming proper activities

Treatments were accomplished by imbibing eggplant and tomato seeds in a solution containing different levels of pyroligneous acid for twenty four (24) hours while radish and cucumber seeds for sixteen (16) hours in darkness under room temperature.

Fifteen (15) petri dishes lined with two (2) layers of tissue papers with 150 seeds each of tomato, eggplant and radish seeds and five (5) petri dishes with *60* seeds of cucumber were saturated with ten (10) ml priming solution based on treatment. Seeds in each petri dish were used per treatment with three replications in the germination test. Following priming, seeds from each petri dish were placed in a sieve and washed with running tap water for 1 minute and left to surface dry in between layers of tissue paper under room conditions for two (2) hours to make separation of seeds easier.

1.1.3 Germination test

Germination test was carried out in a dark cabinet under ordinary room conditions. Seeds were placed in petri dishes lined with two layers of tissue paper moistened with 5 ml distilled water. Treatments were arranged in completely randomized design with three (3) replications having fifty (50) seeds per replicate for tomato, eggplant and radish while 20 seeds per replicate for cucumber seeds.

Germination was monitored and recorded daily until the number of germination was stabilized (for 14 days). Radicle protrusion to 1 mm was scored as germination. Data on initial germination percentage was obtained on the first day of germination when radicle protrusion was observed from each replicate per treatment. From the total number of seeds germinated; speed of germination and final germination percentage were also determined.

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1.2 RESULTS AND DISCUSSION

Based on the data gathered, result indicated that initial germination percentage of radish seeds was accelerated 94% with pyroligneous acid similar with the findings of Yatagai (1987), using wood vinegar from four conifers but not from broad leaf trees, He also found in 1989 that the germination and inhibition effects of alcohol were small or almost nil and the growth of radicle as well as hypocotyl was accelerated by addition of wood vinegar. Considering that pyroligneous acid has strong odor of smoke (smoke water) that contains (3-methyl-2H-furo [2,3-c]pyran-2-one) stimulate and accelerate the germination percentage of radish seeds.

Results showed significant difference on the initial seed germination from treatments 2 and 3 compared to treatments 4 and 5 on the initial germination percentage of radish seeds with mean difference of 53% and 66% from treatments 2 compared to treatments 4 and 5, and 46% and 58 % difference from treatments 3 compared to treatments 4 and 5, respectively Figure 1. (Dixon et al., 2009), said that smoke is a broadly effective stimulant that enhances germination of approximately 1,200 species in more than 80 genera worldwide.

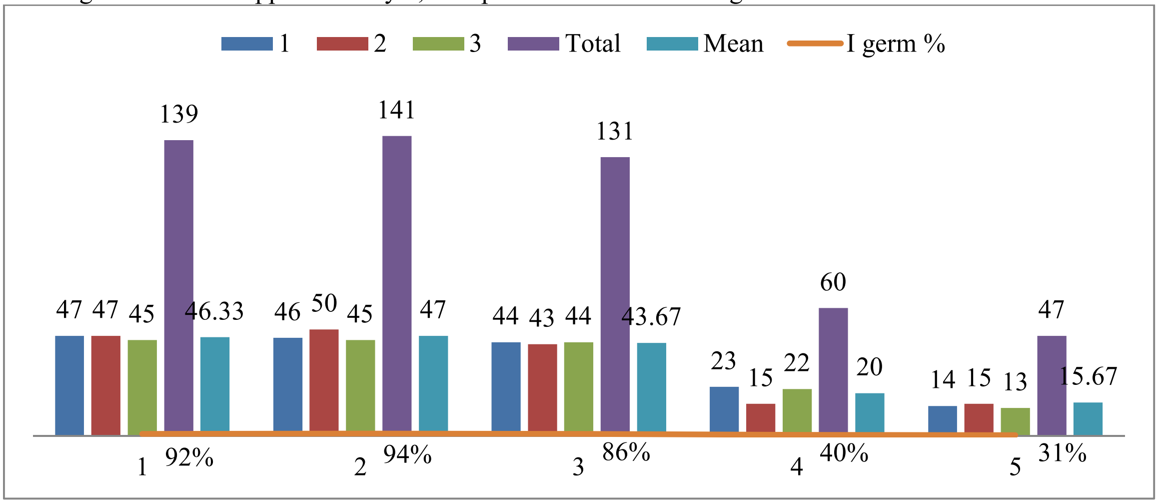

Figure 1. Initial germination percentage of radish seeds with different levels of pyroligneous acid

In terms of final germination percentage (FGP) for radish seeds, one hundred (100) percent germination was observed in all seeds treatments while in terms of the speed of germination (SG) treatment 2 has the fastest rate compared to other treatments, on the other hand no significant difference was observed.

Hoever, highest rate of initial germination on tomato seeds was observed on untreated seeds (T1) followed by seeds treated with 5% pyroligneous acid concentration (T2) and 15% concentration (T4) with mean initial germination percentage of 81%, 78%, and 71% respectively (Figure 2). Results showed no significant difference.

Although, highest initial germination percentage was observed on untreated tomato seeds, the final germination percentage was higher on pyroligneous acid treated seeds three days after the first germination was recorded. Seeds treated with 15% PA (T3), 5% PA (T2), 15% PA (T4) and 20% PA (T5) had 100%, 97% and 96% final germination percentage compared to untreated seeds with only 93% germination.

Ishii (1990) examined germination of vegetables by each treatment of wood vinegar which were obtained separately on the temperature, at which gases where fractional and he found out that wood vinegar promote seed germination.

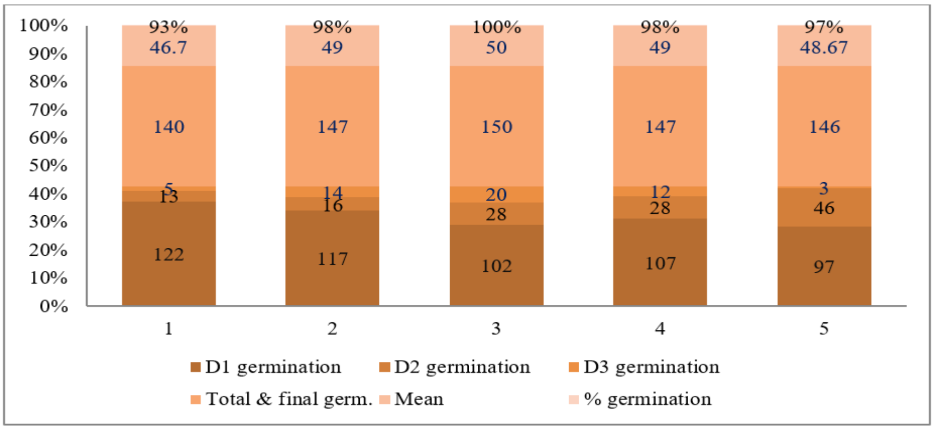

Figure 2. Final germination percentage of tomato seeds with different levels of pyroligneous acid.

On the other hand, one hundred percent germination of cucumber seeds was observed on all treated and untreated seeds with pyroligneous acid. Insignificant difference on the speed and final germination percentage of cucumber seeds were observed.

The effect of pyroligneous acid on the initial germination of eggplant seeds started on the 5th day after the treatment, highest germination rate was observed on the 6th day from T4 (15% PA) with 55% germination compared to other treatments. On the same day, the lowest germination percentage was noted on (T5) 20% PA concentration with mean germination of 7% figure 3.

Data on final germination percentage revealed that eggplant seeds treated with 5% PA (T2), 10% PA (T3), and 15% PA (T4) gave the best results in terms of germination of eggplant seeds compared to untreated seeds, with 99%, 100% and 96% final sprouting percentage, however, 20% PA (T5) inhibit the germination of eggplant seeds with the lowest germination percentage of 76% figure 3. No significant difference on both germination percentage and final germination was observed.

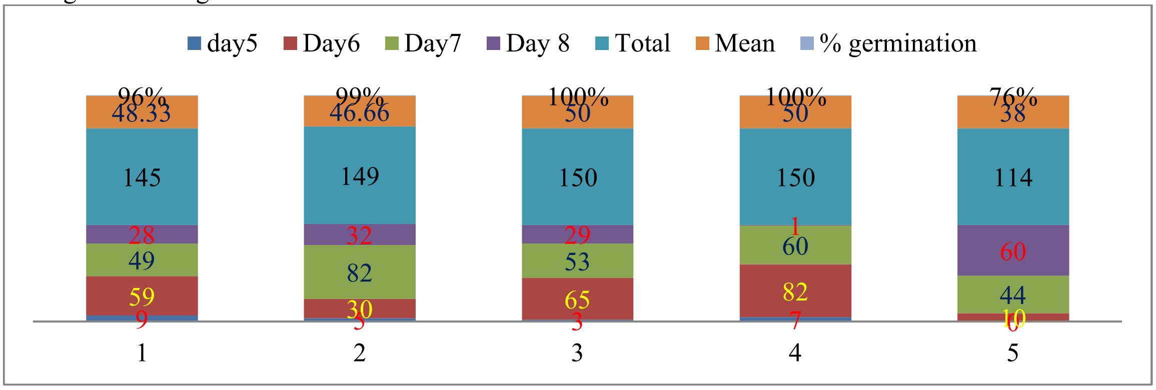

Figure 3. Final germination percentage of eggplant

1.3 SUMMARY

On the initial germination, T2 (5% PA) favor the germination of radish seeds with 94% compared to other treatments. All tomato seeds treated with pyroligneous acid on the other hand, were comparable to untreated seeds in terms of speed germination and final germination percentage. Treatments with 10 and 15% concentrations (T3 and T4) had the fastest rate of germination on eggplant seeds compared to untreated seeds, while T5 (20% PA) had the lowest germination percentage table 1.

Initial germination Percentage of four kind of vegetable seeds					
seeds	T1	T2	T3	T4	T5
eggplant	6.00	3.33	2.00	4.67	0
tomato	81.33	78.00	68.00	71.33	64.67
radish	92.67	94.00	87.33	40.00	28.00
Cucumber	91.67	90.00	85.00	78.33	75.00
Speed of germination of four kind of vegetable seeds					
seeds	T1	T2	T3	T4	T5
eggplant	10.46	8.73	10.82	11.59	6.68
tomato	35.17	41.67	38.67	40.33	40.00
radish	48.83	48.50	46.83	35.00	32.00
Cucumber	19.17	19.00	18.50	17.83	17.50
Final germination percentage of four kind of vegetable seeds					
seeds	T1	T2	T3	T4	T5
eggplant	46.67	49.67	49.33	49.67	36.33
tomato	90.00	88.67	86.67	90.00	95.33
radish	100	100	100	100	100
Cucumber	100	100	100	100	100

Table 1. Summary table on germination of four different vegetable seed cultivars

1.4 CONCLUSION

Priming enhance the germination of tested vegetable seeds (germination percentage of seeds is only 85% Keystone Seed house Corporation) ranging from 93 to 100% germination in all treatments of all seeds except for 20% PA (T5) in eggplant seeds with only 76% final germination percentage.

Pyroligneous acid in the priming solution could speed up the initial germination of radish and cucumber seeds and could increase the germination of tomato and eggplant seeds by 7% and 4 % compared to untreated seeds.

1.5 RECOMMENDATION

Follow-up studies on the different number of days of seed priming should be conducted to determine which could give optimum results in terms of germination of seeds. Studies dealing with the seedling emergence, vegetative, and reproductive stage may be conducted to fully explore the potential of pyroligneous acid as organic fertilizer and growth enhancer.

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