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Influence of Plant-gleaned Compounds on the Initiation and Development of Fungal Diseases of Onion (Allium cepa L.) in the Field

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Abstract
A study on the control of fungal diseases of onion (*Allium cepa* L.) plant; was carried out in the Research and Training (R&T) Farm; of the Michael Okpara University of Agriculture, Umudike, Nigeria. The experiment was a 2 x 3 factorial, laid out in a randomized complete block design (RCBD) with 5 replicates. The treatments comprised of 2 leaf extracts from *Azadirachta indica* and *Dennettia tripetala*; sterile water (control); and 2 periods of application of the test plant-derived compounds: 4 weeks after transplanting (4 WAT) and 6 weeks after transplanting (6 WAT). The fungal diseases identified on the naturally infected onion farm were anthracnose (*Colletotrichum gloeosporioides*), black mold (*Aspergillus niger*), bulb rot (*Rhizopus stolonifer*) and leaf spot (*Alternaria porri; Curvularia sp*). Plots treated with *Dennettia tripetala* and *Azadirachta indica* were significantly (p=0.05) superior to the control plot in the reduction of incidence and severity of these fungal diseases. All the plants treated at 4 WAT with the test extracts performed better in their growth parameters, and yield than those spray-treated at 6 WAT. Plants sprayed at 4 WAT with *D. tripetala* gave the highest mean yield of 11,984.30kg/ha, while those sprayed with sterile water gave the lowest yield of 7,000.56 kg/ha. Therefore, extracts from *Azadirachta indica* and *Dennettia tripetala* should be applied as prophylactics at or before disease initiation (4 WAT) for management of soil and thrash-borne fungal diseases of the onion crop in integrated diseases management programmes in sub-Saharan Africa (SSA) for improved onion production.

INTRODUCTION

Onion (*Allium cepa* L.) (*Liliaceae*), also known as the bulb onion or common onion is the most widely cultivated species of the genus *Allium* (Brewster, 1994; Fritsch and Friesen, 2002), whose origin is thought to be South-West or Central Asia (Jones and Mann, 1963). The crop is a shallow rooted, biennial monocotyledon; that is usually grown as an annual vegetable, in a wide range of climatic conditions (Brewster, 1994). One of the advantages of this spice crop is that the bulbs can be harvested and sold green for salads, while the mature bulbs are cooked as part of the main dish or eaten raw as a vegetable (Straub and Emmett, 1992; Lannoy, 2001). The crop is rich in vitamins, minerals and other nutrients. One hundred grams (100g) of the bulb is reported to contain carbohydrate 9.4g, energy 166 KJ, sugars 44 g, fibre 1.7 g, fat 0.10 g, protein 1.10 g, vitamin C 7.4 ng, potassium 146 ng, phosphorus 24 ng among other vital nutrients. The crop also contains many important phytochemicals such as flavonoids, phenolics, allicin, quercitin, quercitin 3, 4-diglucoside and quercitin 4-glucoside. These phytochemicals are responsible for the distinctive flavour of the crop which is appreciated by people throughout the world. Its oil is especially rich in allyl-propyl-disulphide (4, 5-dithia-1-octene) which is reported to underpin its pungency and spicy attributes in foods and salads; and noted to contribute to the insecticidal, antibacterial and antifungal activities of the crop (CDC, 2011; Roopa et al., 2014).

Average yield of 51.90 million MT of onions are produced on a world scale per annum on 2.97 million ha of farmland. In Ethiopia 13.30 tons/ha from the crop has been documented (Shiberu et al., 2013; Roopa et al., 2014); while a slightly lower value of over 1.20 tons/ha is recorded from the crop in Nigeria. This ranks Nigeria as the 14th largest producer of the crop in the world. Cultivation of the crop in the counry is done in two marked seasons. The rainy season (April-September) and dry season (September/December); though, a mid-season crop (June and November) could sometimes be feasible. Onion production is basically done in northern Nigeria; however, Ayodele (1996) and Awurum (1999); have demonstrated that its large scale production in the South-West and South-East regions of the country is feasible. Awurum (1999); observed that the irrigated crop has yield advantage over the rain fed production system. However, bulbs from irrigated systems are more prone to postharvest rots under normal conditions (Biswani et al., 2010).

Besides thrips, environmental and cultural constraints; soil and thrash-borne fungal diseases especially Purple blotch (*Alternaria porri*), leaf blight (*Alternaria alternata*), black mould (*Aspergillus niger*), blue mould (*Penicillium spp*.), downy mildew (*Perenospora destructor*), twister (*Colletorichum gloeospoiriodes; Gibberilla moniformis*), smudge *(Colletotrichum circinans*), white rot (*Sclerotium rolfsii*), smut (*Urocystis cepulae*), anthracnose (*Colletotrichum spp*.) etc. challenge sustainable onion production and storage in a wide range of agro-ecologies (Montes-Belmont and Prados-Ligero, 2006; Ramjegathash et al., 2011; Gupta et al., 2012; Sibi et al., 2013; Alberto, 2014). These diseases drastically reduce onion productivity, quality and yield. For instance,

yield reductions of 10-50% have been reported in different onion cultivars in Sri Lanka due to bulb rot (Rajapakse et al., 2002); and 30-50% estimated from field and storage as a result of purple blotch in the Americas (Colostate, 2014). Roopa et al (2014) reported that species of *Penicillium*, *Botrytis* and *Aspergillus* participate in up to 25-30% of postharvest spoilage of onion bulbs in India. Besides, some of these mycobiota are mycotoxigenic. Thus there is substantial risk of contamination of the produce with mycotoxins from these species (Enyiukwu et al., 2014a). For example, *Penicillium sp*. produces the toxin Penitrem A. This toxin has recently been implicated in tremogenic toxicosis reported Ara et al. (2008).

Control of these diseases has been achieved in some measure through improved field sanitation, early planting, and avoidance of flooded areas, proper row orientation, and use of resistant varieties. Though sprays with synthetic chemical fungicides such as Bavistin, captan, Indofil M-45, thiram, and Topsin M in field or store could control pathogenic fungal disease in onion; field sprays are preferred (Rajapakse et al., 2002; Gupta *et al.,* 2012). In a trial n Sri Lanka, carbendazim applied 2 weeks before harvesting reduced bulb rots by 40% in the crop (Rajapakse et al., 2002). However, these chemical treatments are accompanied by some poisonous residues in the environment and treated produce. This could have spill-over effects in the food chain that may adversely affect humans in the long run (Enyiukwu et al., 2014a; 2014b; 2014c). Thus, giving impetus for search for alternative pesticides especially of plant origin (Amadioha, 2002: 2003).

Recent evaluations authenticated that higher plants are veritable sources of fungitoxic compounds useable in integrated disease management (IDM) programmes against phyto-fungal diseases (Enyiukwu et al., 2014b). For instance, extracts from *Eucalyyptus tertonia* and *Azadiachta indica* effectively impeded the soil-borne fungal disease black mould (*Aspergillus niger*) of onion in the field (Gupta et al., 2012). Similarly, two divided spray-doses of 3% neem oil at the on-set and fortnight later, retarded the development, spread and disease index of leaf blight (*Aternaria alternata*); strongly improving the crop performance and yield (Ramjegathesh et al., 2011). These plant extracts are non-toxic, cheap, easier to obtain and prepare, accessible to farmers and contain multiple bioactive secondary metabolites which make pathogens' resistance to them unlikely in the long-run (Dominic *et al.,* 2005, Enyiukwu *et al*., 2014a; 2014b; 2014c).

This work therefore evaluates the influence of compounds gleaned from some tropical plants (*Dennettia tripetala, Azadirachta indica*) on the fungal diseases of onion in the field; and impacts of these treatments on the performance and yield parameters of the crop.

MATERIAL AND METHODS

Source of Materials

The experiments were conducted in the Crop Science Laboratory and the Research and Training Farm, of the Michael Okpara University of Agriculture, Umudike; in the humid rainforest agro-ecological zone of South-East Nigeria. The seeds of the onion variety Rouge De Tana (Technisem, France) obtained from Agritropic Ltd., Kano Nigeria; and leaves of *Dennettia tripetala*, and *Azadirachta indica* collected from Umudike were used for the study.

Preparation of plant extracts and culture medium

Then the leaves of *Dennettia tripetala* and *Azadirachta indica* collected from the neighbourhood, were washed in several changes of tap water and air-dried on the laboratory bench for 20 days at 27°C. Thereafter they were milled into powder with a Thomas Wiley milling machine (Model: ED-5 USA) (Awurum and Okorie, 2011). Thirty grammes (30g) of each powdered plant specimen was then soaked separately in a 250 ml beaker containing 100 mL sterile water, stirred vigorously and then allowed to stand for 1 h (Amadioha and Obi, 1999). The suspensions were sieved through a double fold cheese cloth to obtain 30% strength of the respective extracts. Twenty grammes (200g) of washed, peeled Irish potato was boiled in 500 mL sterile distilled water for 1h. The broth was sieved through double fold cheese cloth into a 2 L flat bottom flask and made up to 1000 mL with sterile distilled water. Then 20 g of agar powder and 15g of glucose were added to it and modified with 20mg of Gentamicin (an antibiotic to kill or suppress any bacteria contaminating the medium); stirred vigorously with a glass stirrer and covered with foiled stopper, and autoclaved at 15 pounds pressure (120°C, 154cmHg) for 30 minutes.

Field experiment

The field measuring 85m^2 (17 x 5m) was slashed, ploughed, harrowed and manually prepared with seed bed of 1m x1m. The seedlings were raised by broadcasting at a groove from seeds mixed with river sand and mulched with grass straw. The nursery was watered once daily. Two weeks before transplanting poultry manure was worked into the beds at 10 tons/ha, and irrigated daily to enable the manure decompose. The beds were further broadcast-improved with inorganic NPK fertilizer (15:15:15) at 250 kg/ha 24 h before transplanting. At 5 weeks after planting the seedlings were transplanted on to the beds at a spacing of 20 cm x 20 cm. The experiment was laid out as a 2 x 3 factorial with five replicates, in a randomized complete block design (RCBD). The beds were

manually weeded every fortnight, beginning from 2 weeks after transplanting (WAT). At 4 WAT and 6 WAT the onion plants were sprayed with the extracts thus prepared from leaves of *D. tripetala* and *A. indica*, using a hand sprayer.

Six plants at the middle rows were tagged and data on plant height, girth, types, incidences and severity of different fungal diseases were taken from them. The bulbs were also harvested at 4 months after transplanting, cured for 7days and weighed with a digital balance. The % incidence of the disease on the inoculated onion seedlings was assessed using the formula by Olufolaji (1999) as:

$$\% \text{ incidence} = \frac{\text{Number of Plants with Disease} \times 100}{\text{Total Number of Plants Examined} \quad 1}$$

The % severity of the disease was taken by visual assessment of the test plants with typical symptoms of the fungal diseases, using the descriptive scale outlined by James (1983) as modified:

1 - No symptom
2 - Lesions present on less than 20% tissue
4 - Lesions present on less than half of the tissue
6 - Lesion present on up to 60% of the tissues
8 - Lesions present on most of the tissues
10 - Heavy lesions on tissues, defoliation occurs.

And calculated; according to the formula adopted from James (1983) as:

$$\text{Disease severity} = \frac{\text{Sum of individual disease ratings}}{\text{Total number of plants examined}}$$

The fungitoxicity of the extracts was evaluated as the reduction in severity of the fungal diseases on the plants sprayed with the test extracts in the naturally infected onion field; compared to the controls (water or benomyl).

Isolation and Identification of the Causal Pathogens

Diseased onion (*Alium cepa* L.) tissue collected from the sprayed onion field were cut into pieces and surface-sterilized in 70% alcohol and then rinsed in three changes of 300 mL sterile distilled water. The tissues were then plated in Petri dishes containing moistened Whatman No 1 filter paper, covered and incubated for 5 days, and observed for fungal growths. The mycelial growth from the plated tissues was subcultured repeatedly to obtain pure culture of the organisms which were maintained on PDA already prepared. Slides of the organism were mounted and examined under a microscope. The organisms' identities were individually confirmed by the aid of identification manual by Barnett and Hunter (1995).

STATISTICAL ANALYSIS

Data collected were analyzed by ANOVA using the general linear model procedure in SAS system (2008 version) at significant level of 5%. Means were separated and compared using Fishers Least Significant Difference (FLSD) at probability of 0.05.

RESULTS

The diseases identified on the onion plants were anthracnose (*Collectotrichum gloeosporioides*), black mold (*Aspergillus niger*), bulb rot (*Rhizopus stolonifer*) and leaf spot (*Alternaria porri; Curvularia sp*) (Table 1). It indicated that anthracnose, followed by Alternaria leaf spot were the most frequently occurring diseases on the trial onion farm.

The results also indicated that the test extracts (*Dennettia tripetala* and *Azadirachta indica*) effectively impeded the initiation, growth and development of these diseases on the onion plants. The percentage incidence and severity scores of these diseases on the naturally infected trial plots, reveal that these parameters were lower on plants treated with the phyto-chemicals at 4 WAT (Figure 1; 3) than those exposed to the same treatments at 6 WAT (Figures 2; 4). Furthermore, onion plants exposed to the phytochemical treatments performed better in terms of growth parameters (height, number of leaves and girth diameter) (Table 2), and bulb yield (Table 3).

Results obtained on the yield of *Allium cepa* were presented in Table 2. Generally, there were significant difference ($P < 0.05$) between the plant treated at 4 WAT and those treated at 6 WAT irrespective of the extracts applied. The mean bulb yield of onion plants treated at 4WAT and 6WAT were 11,984.30kg/ha and 10,829.70 kg/ha respectively. In terms of reduction of the incidence and severity of the pathogens-induced lesions, phytochemicals from *Azadirachta indica* and *Dennettia tripetala* were significantly ($p=0.005$) superior than sterile water. Plants from plots treated with *Azadirachta indica* gave mean bulb weight of 11,009.10kg/ha, while *Denniettia tripetala* and sterile Water gave 11,984.90kg/ha and 7000.56kg/ha respectively (Table 3).

DISCUSSION

Several fungal diseases have been reported to attack onion plants and/or bulbs in the field, transit or store. The

association of *Aspergillus niger, A. flavus, A. fumigatus, Alternaria porri, Rhizopus stolonifer, F. oxysporium, Penicillium citrinum, Sclerotium cepivorium, Colletotrichum gloesporioides* and *Giberella moniformis* amongst other diseases with the crop have been documented (Montes-Belmont and Prados-Ligero, 2006; Gupta *et al*., 2012; Sibi *et al*., 2013; Alberto, 2014). Our findings of the association of members of the genera *Colletotrichum, Aspergillus, Rhizopus, Alternaria* and *Curvularia* with the crop agree with these reports.

Several authorities have asserted the toxicity of plant-derived compounds to fungal pathogens of onion crop. Two divided doses of 6% neem oil, for instance, applied fortnightly impeded the development and spread of onion leaf blight (*A. porri*) in the field (Ramjegathesh et al., 2011). Reports of extracts from *Syzygium aromaticum, Cinnamonium zeylanicum* and *Paihyrrizus erosus* impeding the growth and sclerotial production by *S. cepivorium* (white rot) is well documented (Montes-Belmont and Prados-Ligero, 2006). Gaikwad et al. (2014) demonstrated that *Lawsonia alba Moringa oleifera* and *Parthenium hysteophorus* retarded the growth of *A. porri, F. oxysporium* and *Stemphlium vesicarium* isolated from onions in culture. In a parallel field study, phytochemicals from *Dennettia tripetala* and *Spondias mombin* significantly reduced the incidence and severity of wet rot of amaranthus vegetable caused by *Choanephora curcubitarium* (Awurum and Nwaneri, 2011; Awurum and ogbonna, 2013). The findings from these investigators also harmonize with ours.

The plant extract treatments were more effective when applied at 4 weeks after transplanting (WAT) than at 6 WAT. This further lends support to similar assertions from our previous studies that same period of control application was superior in retarding the initiation and development of fungal diseases in groundnut (*Arachis hypogoea* L.) and *Amaranthus cruenta* L. to administering same treatments at 6 weeks after transplanting (Awurum and Nwaneri, 2011; Awurum and Ogbonna, 2013; Awurum and Uchegbu, 2013). It suggests therefore, that these fungal diseases are more virulent during the first 4 weeks of growth of the onion plant; a view also held by Awurum and Uchegbu (2013) in the parallel study. Recently, Alberto (2014) reported a correlation between declining levels of sugar, protein and phenols to decreased resistance of onion plant to anthracnose and twitter disease attacks. The author emphasized that low amounts of phenols encouraged increased susceptibility of the crop to these infections.

Phenols are aromatic alcohols, which are constituents of various ranges of pesticides. In bio-systems this class of phytochemical has been reported to block cell division, slow cellular growth and elongation, hamper sporangia formation, spore development and impair a wide array of microbial enzymes. (Enyiukwu and Awurum, 2013). Applying phytochemical controls early enough in this study, may have supplied phenols to, or improved the production of infection-fighting phenolics in the test onion plants; and thus reduced the advancements and damage due to these fungal diseases. Hence arresting the diseases at the earlier stages (4 WAT) translated to the improved performance of the crop (Table 2); evidenced in the superior bulb yield than those exposed to the same treatment at 6 WAT (Table 3).

The superior fungitoxic activity of *D. tripetela* over *A. indica* connotes that this phytochemical may contain larger amounts of phenolic compounds and/or that these phenolics are more easily liberated in the presence of stimulating toxins from the onion pathogens than those of *A. indica*. Or that the *D. tripetaa* phenolics are more tolerant to environmental stressors of temperature and UV radiation.

In conclusion therefore, our findings from this work support that farmers should use the extracts of *Dennettia tripatala* and *Azadirachta indica* (especially *D. tripetalla*) to control fungal disease of onions. And that the application should be administered early at the on-set or before disease initiation to prevent the pathogens from causing much damage to the crop; thereby improving onion production.

4 WEEKS AFTER TRANSPLANTING (WAT)

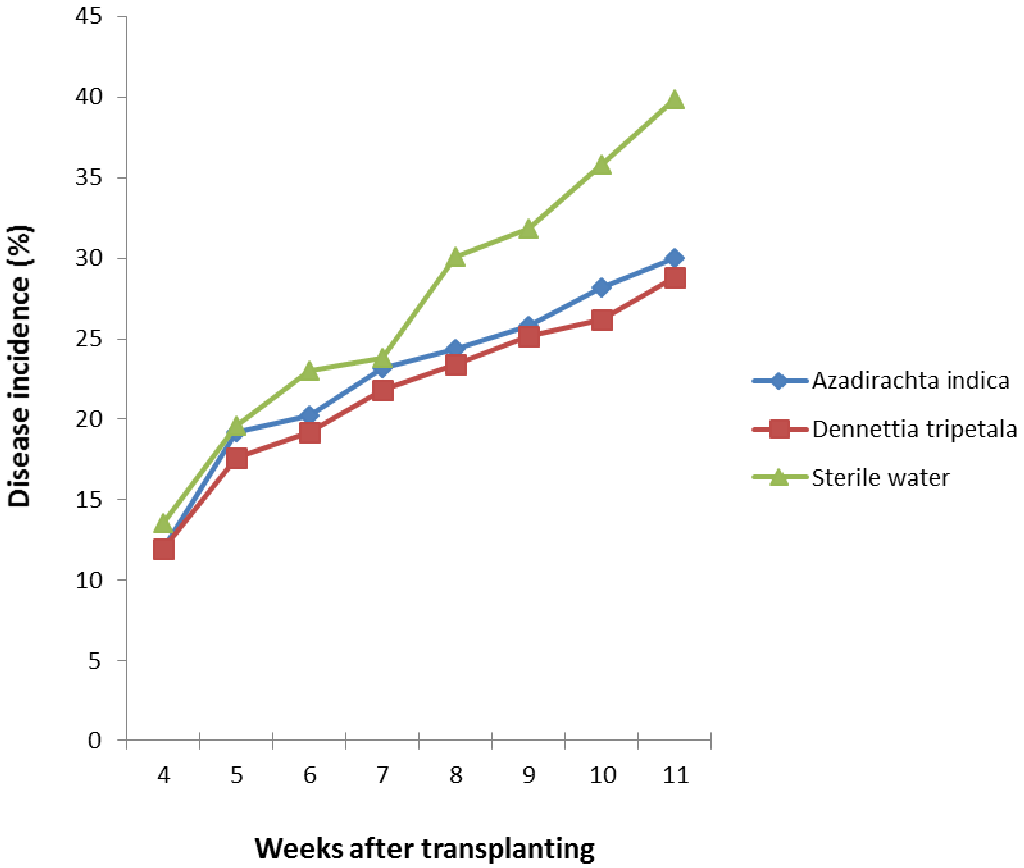

Figure 1: The progress curve of disease incidence showing the effect of the extracts at 4 Weeks spray regime.

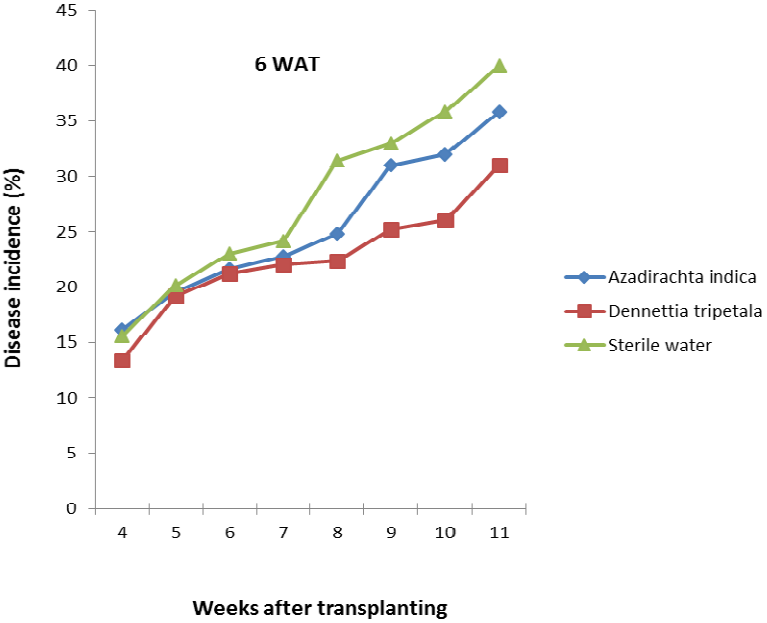

Figure 2: Progress curve of disease incidence showing the effect of the extracts on the disease incidence at 6weeks spray Regime.

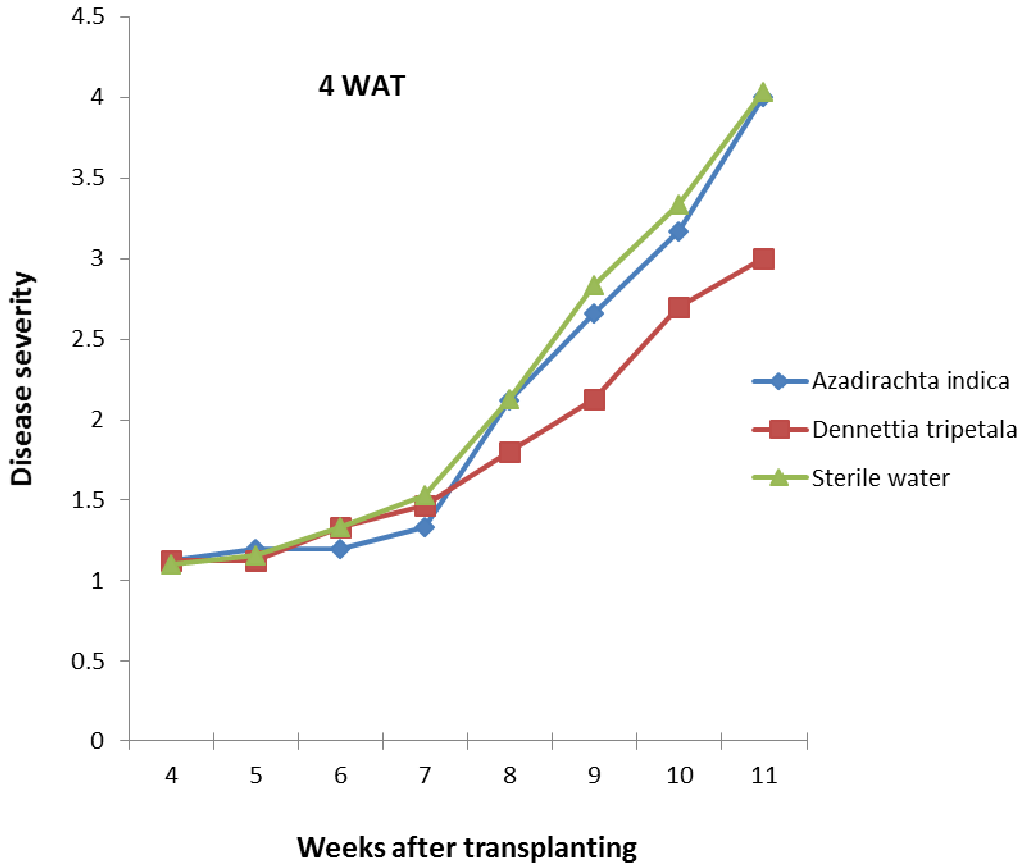

Figure 3: Progress curve of disease severity showing the effect of the extract on the disease severity at 4 weeks spray Regime.

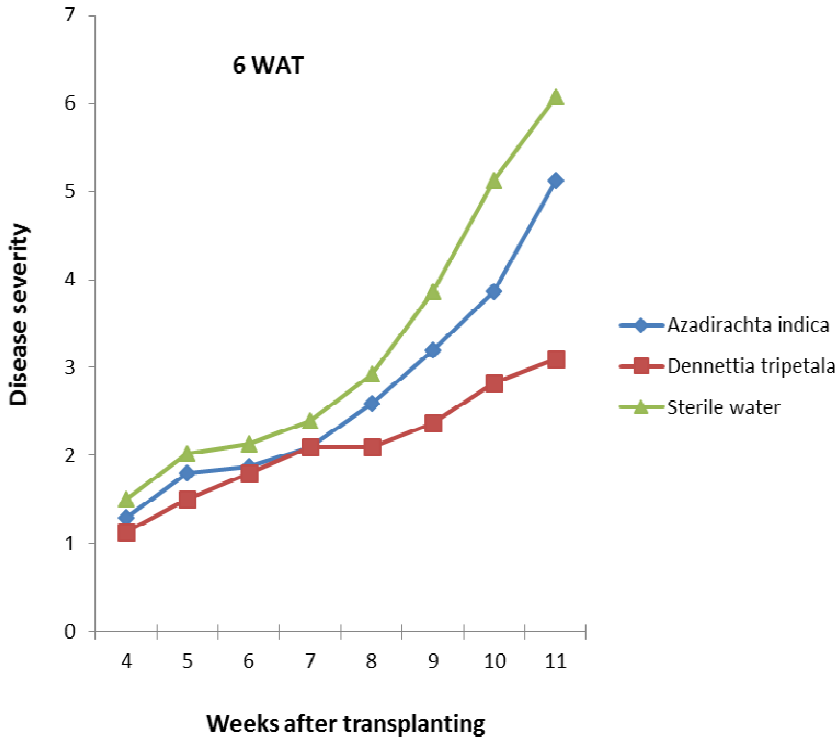

Figure 4: Progress curve of disease severity showing the effect of the extracts on disease severity at 6weeks spray Regime.

Table 1: Onion diseases and their frequency of occurrence in the trial farm.

Disease/Mycobiota	Frequency of disease occurrence (%)
Anthracnose (*Collectotrichum gloeosporioides*)	38.20
Black mold (*Aspergillus niger*)	11.37
Bulb rot (*Rhizopus stolonifer*)	11.95
leaf spot (*Alternaria porri*)	29.06
leaf spot (*Curvularia sp*)	09.42

Table 3: Yield of *Allium cepa* L. (Kg/ha) obtained in the experimental field.

Treatment Extract of	Application Period 4 WAT	6 WAT	**Mean Extract Effect
Azadirachta indica	11571.40	10446.80	11009.10
Dennetia tripetala	12397.20	11212.60	11804.90
***Mean Yield A/Period**	**11984.30**	**10829.70**	**--**
Sterile water	7000.90	7001.21	7000.56

*** LSD (Yield): 900.88; **LSD (Extract): 582.13**

Table 2: Influence of the phytochemical treatments on the growth parameters of *Allium cepa* L. plants.

Treatment	Weeks after transplanting (WAT) 4			5			6			7		
	PH (cm)	NL	GT(cm)	PH(cm)	NL	GT(cm)	PH(cm)	NL	GT(cm)	PH(cm)	NL	GT(cm)
Extract sprayed at **4 WAT**												
A.I	35.33	4.79	1.06	36.33	4.83	1.23	40.72	5.83	1.32	40.58	5.93	1.39
D.T	37.00	4.83	1.09	39.63	5.10	1.32	39.37	5.77	1.41	39.77	5.86	1.44
S.W	34.83	3.48	0.93	35.09	3.56	1.00	37.67	4.27	1.14	37.70	4.20	1.22
Extract Sprayed at **6WAT**												
A.I	32.96	4.13	0.98	33.10	4.20	1.27	36.62	4.61	1.25	38.64	5.36	1.35
D.T	37.11	4.20	1.10	37.97	4.36	1.24	42.56	4.70	1.36	43.20	5.55	1.38
S.W	32.33	3.29	0.83	32.43	3.13	1.01	35.03	4.09	1.09	36.40	4.19	1.22
LSD (=0.05)	**5.24**	**0.61**	**0.22**	**4.86**	**0.51**	**0.20**	**5.90**	**0.67**	**0.20**	**5.17**	**0.58**	**0.19**
	4.28	**0.50**	**0.18**	**3.97**	**0.42**	**0.16**	**4.81**	**0.55**	**0.17**	**4.22**	**0.48**	**0.16**

*A.I = *Azadirachta indica,* D.T = *Dennettia tripetala*, S.W = Sterile water, PH = plant height (cm), NL = Number of leaves, GT=Girth (cm)

Plate1: *Allium cepa* plot treated with *Denniettia* tripetala at spray regime of 4 WAT as at the 8 WAT with no significant Symptoms of diseases

Plate 2: *Allium cepa* Control plot (sterile water treated) at 8 WAT showing high level of defoliation and symptoms of diseases.

Plate 3: Harvested *Allium cepa* bulbs during Curing on the laboratory bench of the Crop Science Laboratory.

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