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Full Length Research Paper

Role of *Dirhinus giffardii* Silv. age on the parasitism preference to different days old pupae of *Bactrocera zonata* and *Bactrocera cucurbitae*

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Apprehensions are mounting about the effects of pesticides on human and environment. Therefore, interest is being revived to use biological control, which is recognized as an important component of sustainable pest management. *Dirhinus giffardii* has been effectively used as pupal parasitoid for the management of fruit flies. Experiments were conducted to determine the suitable host age for rearing of the pupal parasitoid on the two species of fruit flies, *Bactrocera zonata* and *Bactrocera cucurbitae*. Results indicated that *D. giffardii* preferred the pupae of *B. zonata* than *B. cucurbitae* at all the tested host (pupae) ages of the fruit flies. Maximum parasitism per female was observed at the age of 3 days old pupae. The parasitism increased significantly up to the pupal age of 3 days and then the parasitism started declining. Parasitism of the *D. giffardii* was recorded on fruit flies pupae up to the age of 5 to 6 days on both the fruit fly species and no parasitism was recorded from day 7 onwards. The results revealed that age of parasitoid, *D. giffardii* also had significant effect on pupal parasitism of *B. zonata* and *B. cucurbitae*. The mean parasitism per female was increasing with age of parasitoid and reached to its peak at the age of 5 days of parasitoids. Thereafter the parasitism started declining with the subsequent age of parasitoid and it reached lowest at the age of 30 days. The studies suggested that the parasitoids *D. giffardii* should be discarded after the age of 15 days for good mass rearing.

Key words: Pupal parasitoids, fruit flies, host and parasitoid ages, parasitism.

INTRODUCTION

The insect pests adversely affecting agricultural productions are commonly controlled by the application of large quantities of pesticides. Production is affected in the field, prior to harvest, and after harvest. In general, globally 30 to 40% losses in field and post harvests by the insects are common (Mathew, 1999). Due to frequent and

injudicious application of pesticides insect pests develop resistance that jeopardized their efficiency and also deteriorate the environment. (Van Emden et al., 2004). Mainly the urban public is fervently more disparate to the present control strategy of insect pests in the field crops and the fruit orchards. Further, concerns regarding the

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impact of poisonous chemicals on biodiversity and the environment in particular have augmented the necessity of implementing non-insecticide control programs. Thus it is imperative to search for the pest control strategies that would reduce the quantity of cruel and wide-range usage of pesticides for suppression of the agricultural pests. With expression focused on alternative control program, there has been a renewed interest in biological control. Appropriate application of biological control tends to be effective, environmentally safe and sustainable approach for pest management. Releases of the natural enemies at appropriate stage and time in the field are another critical component for successful application of biocontrol technology (Van Lenteren et al., 2006).

Fruit flies (Diptera: Tephritidae) are very common pests of economic importance in nearly all tropical, subtropical and various temperate regions of the world (De Meyer et al., 2010). The cosmopolitan nature of fruit fly species highlights their international importance in sustainable fruit and vegetable production as well as trade issues. Most of the economic species of fruit flies (*Bactrocera zonata*, *Bactrocera dorsalis*, *Bactrocera cucurbitae*, *Dacus ciliatus*) are polyphagous in nature and damage a wide range of fruits and vegetables affecting their production (Imran et al., 2013). High value exports of fruit (citrus, guava, and mango) and vegetables significantly contribute in the national economy of Pakistan (Anonymous, 2009). To disinfest the fruits, expensive quarantine treatments are often a prerequisite for such exports that is, long duration cold storage, heat treatment, controlled atmospheres, irradiation; such post-harvest tactics increase the expenditure and ultimately reduce quantity of the products. Generally, organophosphate insecticides are recommended to control fruit flies in Pakistan (Mian et al., 1986). In recent decades, biological control offers one of the most promising, environmentally sound, and sustainable tools for control of arthropod pests (Van Driesche et al., 2008). It is therefore, imperative that biological control also be exploited for the management of fruit flies in Pakistan to the fullest extent.

The importance of parasitoids in the augmentative release of biological control of many pests has been reported by various workers (Wang and Messing, 2004). The fruit flies pupal parasite, *Dirhinus giffardii* (Hymenoptera: Chalcididae) has the potential to be exploited as bio-control agents against different fly species of Pakistan but its parasitism on different hosts may be variable and needs to be determined. Dresner (1954) reported that *D. giffardii* could attack *B. dorsalis* puparia previously parasitized by *Fopius vandenboschi* in Hawaii and has only a slight preference for un-parasitized over parasitized puparia. However, detailed information is lacking on potential interactions between *D. giffardii* and other principal fruit fly parasitoids. Some aspects of the biology of *D. giffardii* have been documented by Dresner (1954), Podoler and Mazor (1981). Sangvorn et al. (2004) performed laboratory investigations on the pupal parasitoid (*Spalangia endius* Walker) of fruit fly

Bactrocera correcta (Bezzi) and *B. dorsalis* (Hendel). They mainly find out the effect of parasitoid age, pupal age and host-parasitoid density on the rate of parasitism and reported the peak of parasitism by the females at the age of 3 days. The rate of parasitism of *B. dorsalis* was in the increasing order turned down to below 50% with the pupae age of 7 days, while that of *B. correcta* remained above 90%. Their studies on varying host density revealed that the numbers of parasitized pupae increased with host bulk, but the percentage parasitism went on the decline and was inversely density dependent. In the experiments on variable host (or parasitoid) density, the percentage parasitism was significantly higher in *B. correcta* compared to *B. dorsalis* at all densities they tested. As *D. giffardii* is a very important parasitoid, therefore, the present studies were planned to evaluate the comparison of parasitism and development of the parasitoid at different parasitoid and host ages on the pupae of *B. zonata* and *B. cucurbitae*, the two economical and predominant fruit fly species of Pakistan.

MATERIALS AND METHODS

The experiments were conducted at nuclear institute of agriculture (NIA), Tando Jam. The parasitoids and pupae of the two fruit fly species, *B. zonata* and *B. cucurbitae* were obtained from their respective colonies being maintained at NIA for the last several years. The fruit flies, *B. zonata* and *B. cucurbitae* were mass cultured on sugar, water and protein hydrolysate. On the pop out day, the trays were kept in pupation substrate in the large trays to collect the pupae. By this method, the pupae of the same age were collected through sieving the pupation substrate daily and used for experimentations. The strains of pupal parasite, *D. giffardii* is also being maintained at NIA fruit fly rearing laboratory since the last four years. The parasitoids are being mass cultured on the pupae of *B. zonata* for releases in the guava and mango orchards against fruit flies. The parasitoids used in the studies were cultured on *B. zonata* pupae and sexed immediately after emergence and used according to the requirement of each experiment. After sexing, the newly emerged parasitoid wasps were held in screen cage separately at $25 \pm 2^\circ\text{C}$, 60% R.H, 10:14 D/L and provided with water and honey. All the experiments were conducted under the same environmental condition in which parasitoid and the host pupae were kept.

Effect of age of the host (pupae) for parasitism

A sample of 300 pupae each of *B. zonata* and *B. cucurbitae* were exposed to five pairs of 2 days old parasitoid *D. giffardii* at different ages of the host pupae. For this purpose a stock of 5000 selected pupae of the each fruit fly species were separated carefully from the oviposition substrate on day one just after the larval pop out and kept in plastic containers separately. From the stock culture, 300 pupae of the fruit fly species were offered to the two days old five pairs of the parasitoids on each day starting from 1 to 8 day of their ages for parasitism in $20 \times 20 \times 20$ cm perplex sheet cages having wire screen on one side. To obtain the similar age of the parasite, newly emerged parasitoids were sexed by examining the abdominal

Table 1. Effect of host age on parasitism of *Dirhinus giffardii* on the pupae of *Bactrocera zonata* and *Bactrocera Cucurbitae*.

Pupae offered on day (age)	<i>Bactrocera zonata</i>	<i>Bactrocera cucurbitae</i>
1	12.34 ^d ± 0.45	7.23 ^c ± 0.38
2	31.67 ^b ± 0.68	16.18 ^b ± 0.43
3	43.84 ^a ± 0.82	36.45 ^a ± 1.46
4	24.05 ^c ± 0.46	16.2 ^b ± 0.56
5	7.45 ^e ± 0.43	4.25 ^d ± 0.24
6	0.52 ^f ± 0.12	0.58 ^e ± 0.11
7	0	0
8	0	0
LSD values	1.430	1.753

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through analysis of variance followed by DMRT (P=0.05).

tips of the wasps and kept separately in two perplex cages. The adult wasps were fed with 30% water and honey solution. The pupae were exposed to the parasitoids in the cages have wire netting on one side and the remaining side of the cages is made by transparent perplex glass. Every day 300 pupae of the two fruit fly species with four replications were offered to two day old parasitoids for parasitism for twenty four hours. The experiment was continued up to 8 days of the pupal age. The rate of parasitism on each fruit fly pupae was recorded at the time of emergence of the parasitoid wasps from the pupae.

Effect of the parasite age to parasitize the host pupae

Freshly emerged parasitoids of *D. giffardii* were sexed and kept separately in oviposition cages and fed with 30% water and honey solution. These emerged male and female adult wasps of *D. giffardii* were kept in the laboratory maintained at 27 ± 2°C temperature and 65 ± 5% relative humidity. From the stock culture, 5 pairs of the parasitoids were released at their different ages ranging from 1 to 30 days with 5 time intervals in perplex glass cages measuring 20 × 20 × 20 cm and then they were offered 300 pupae of 2 days old of *B. zonata* and *B. cucurbitae* separately. For each set of the age, fresh un-mated parasitoids pairs were obtained from the stock and they were presented 300 pupae of the respective fruit fly species. The pupae, to each age of the parasitoids were exposed for 24 hrs in four replications and kept separately till emergence of the parasitoid wasps. The data recorded was calculated on parasitism per female basis (parasitoid emergence) at each tested age of the parasitoids starting from 5 to 30 days.

In both set of experiments, four replications were performed for evaluation of the effects. The experiments were conducted in completely randomized design (CRD) and analyzed using the analysis of variance (ANOVA). Duncan's Multiple Range was used to distinguish the means.

RESULTS

Effect of the ages of the host (pupae) on parasitism

The results on the effect of different ages of pupae on the parasitism of *D. giffardii* revealed that the age of pupae played a significant role for parasitism

(Table 1). The parasitism rate increased as the age of the pupae of *B. zonata* and *B. cucurbitae* advanced. The peak of parasitism on pupae of both the fruit fly species were recorded at the age of 3 days and thereafter the parasitism started decreasing gradually. The results showed that on a very first day the mean parasitism by *D. giffardii* on pupae of *B. zonata* and *B. cucurbitae* was 12.34 and 7.23 per female, respectively. The trend was then increased gradually and on the 2 days old pupae of both the fruit fly species, it was 31.67 and 16.18 per female, respectively. The maximum numbers of pupae of the fruit fly species were parasitized at the age of 3 days (43.84 of *B. zonata* and 36.45 of *B. cucurbitae*) and then a decreasing trend in the parasitism rate was investigated with the successive ages of the pupae. The parasitism per female at the pupal age of 4 days of *B. zonata* and *B. cucurbitae* was 24.05 and 16.2, respectively. The same was much reduced when the parasitoids were offered pupae of the age of 5 days (7.45 on *B. zonata* and 4.25 on *B. cucurbitae*). The parasitism reached to negligible level at the age of 6 days of the pupae of both the fruit flies species (0.52 and 0.58, respectively). No parasitism was recorded on the pupae at the age of 7 and 8 days. The present research findings also confirmed that the parasitism rate was relatively much higher on the pupae of *B. zonata* compared to *B. cucurbitae* which suggest that the former is preferred host for the parasitoids and can efficiently be used for mass rearing of *D. giffardii* under laboratory conditions. The parasitoid preferred to attack 2 to 3 days old pupae in which the host pupae had fully established instead of 1 day old pupae. It has been observed that after complete development of the adult in the puparia that is, at the age of 6 day of the host pupae the parasitism by *B. giffardii* was almost negligible and no parasitism was recorded at the pupal age of 7 days onward. An identical trend of age effect for parasitism of *D. giffardii* was recorded on pupae of both the fruit

Table 2. Effect of parasitoid, *Dirhinus giffardii* age to parasitized the two day old pupae of *Bactrocera zonata* and *Bactrocera cucurbitae*.

Age of the parasite (Days)	Mean parasitism per female by	
	<i>Bactrocera zonata</i>	<i>Bactrocera cucurbitae</i>
1	20.90 ^c + 0.24	15.90 ^c + 0.34
5	46.60 ^a + 0.65	37.80 ^a + 0.50
10	28.30 ^b + 1.12	22.35 ^b + 0.48
15	22.15 ^c + 0.40	14.05 ^d + 0.30
20	11.00 ^d + 0.52	5.40 ^e + 0.32
25	3.00 ^e + 0.18	1.35 ^f + 0.17
30	1.50 ^e + 0.13	0.52 ^f + 0.10
LSD values	1.662	0.917

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through analysis of variance followed by DMRT (P=0.05).

fly species tested. However, the parasitism preference by *D. giffardii* was higher on *B. zonata* pupae in comparison to the *B. cucurbitae*.

Effect of parasitoid *D. giffardii* age to parasitized the host pupae.

The results revealed that the age of parasitoid, *D. giffardii* had a significant effect on the pupal parasitism of the fruit fly species, *B. zonata* and *B. cucurbitae*. The mean parasitism per female was in the increasing order with the age of parasitoid and reached to its maximum level at the age of 5 days of parasitoids (Table 2). Thereafter the parasitism started declining gradually with the subsequent age of parasitoid. The results showed that number of pupae of *B. zonata* and *B. cucurbitae* parasitized by *D. giffardii* at the age of 1 day was 20.90 and 15.90 per female, respectively. The peak parasitism by the parasitoids was recorded at the age of 5 days where the mean parasitism of both the fruit fly species was 46.60 and 37.80 per female, respectively. The decreasing trend was observed with further progress in age of the parasitoids and at the age of 10 days of *D. giffardii*, the parasitism on *B. zonata* and *B. cucurbitae* pupae was reduced to 28.30 and 22.35 per female, respectively. The same was much reduced at the age of 15 days of the parasitoids where mean parasitism per female on *B. zonata* and *B. cucurbitae* was 22.15 and 14.05, respectively. The pupal parasitism decreased further at the latter stages of life and the lowest was recorded at the parasitoid age of 30 days (1.5 of *B. zonata* and 0.52 of *B. cucurbitae*). The present research findings confirmed that parasitoids up to the age of 15 days resulted in considerable rate of parasitism of the host pupae with maximum level of parasitism at the age of 5 days. It is also important to mention that rate of parasitism by *D. giffardii* at all ages tested was relatively

higher on the pupae of *B. zonata* compared to *B. cucurbitae* which represent inclination of parasitoids towards the pupae of former fruit fly species.

DISCUSSION

In the present studies *D. giffardii* preferred the *B. zonata* pupae than the *B. cucurbitae*, although *B. zonata* pupae are smaller than the *B. cucurbitae*. These findings are in contradiction with that of Wang and Messing (2004). They observed that body size of the host species showed positive correlation with host size and the parasitoids emerged in case of pupal parasite *D. giffardii*. They reported that the parasitoids consumed almost all the host resource when emerged from the host puparia of either species that is the parasite gained maximum fitness when reared on the larger host. However, they did not observe any effect on the development time of both male and female *D. giffardii* when reared on different sizes of host species. Their studies also showed that *D. giffardii* preferred to parasitize the larger pupae of *Bactrocera litifrons* than to parasitize the pupae of *Ceratitidis capitata*. These findings showed smoothness in the body growth of this generalist parasitoid. It suggests that there was no obvious exchange in the body size and development time in *D. giffardii*, although it may vary in respect to assortment and ecological distinction. There is another possibility that *D. giffardii* can prefer *B. zonata* pupae for parasitism as compare to the *B. cucurbitae* is may be due to the presence of grooves on *B. cucurbitae* pupae, which are more prominent on *B. cucurbitae* pupae. Sangvorn et al. (2004) performed laboratory investigations on the pupal parasitoid (*Spalangia endius* Walker) of fruit fly *B. correcta* (Bezzi) and *B. dorsalis* (Hendel). They observed the effect of parasitoid age, pupal age and host-parasitoid density on the rate of parasitism and recorded the peak of parasitism by the females at the age of 3 days. They reported that the rate

of parasitism of *B. dorsalis* was in the increasing order and turned down to below 50% with the pupae age reached at 7 days old, while that of *B. correcta* remained above 90% at this age. Their studies on varying host density revealed that the numbers of parasitized pupae increased with host bulk, but the percentage parasitism went on the declining trend and was inversely density dependent. In the experiments on variable host (or parasitoid) density, they observed that the percentage parasitism was significantly higher in *B. correcta* compared to *B. dorsalis* at all densities tested. We also observed the peak parasitism per female at the parasitoids age of 5 days on 3 days old pupae. The studies suggested that the parasitoids *D. giffardii* should be discarded after the age of 15 days for the maintenance of good mass rearing colony.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

- Anonymous (2009). Agricultural Statistics of Pakistan 2008-2009. Govt. Pak. Mini. Food, Agric. Livestock (Economic wing), Islamabad.
- De Meyer M, Robertson MP, Mansell MW (2010). Ecological niche and potential geographic distribution of the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae). *Bull. Entomol. Res.* 100(1):35-48. <http://dx.doi.org/10.1017/S0007485309006713> PMID:19323851
- Dresner E (1954). Observations on the biology and habits of pupal parasites of the oriental fruit fly. *Proceed. Hawaiian Entomol. Soc.* 15:299-310.
- Imran R, Ahmad N, Rashdi SMMS, Ismail M and Khan MH (2013). Laboratory studies on ovipositional preference of the peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) for different host fruits. *Afr. J. Agric. Res.* 8(15):1300-1303.
- Mathew BT (1999). Ecological approaches and the development of "truly integrated" pest management. *Proc. Natl. Acad. Sci. USA* 96:5944-5951. <http://dx.doi.org/10.1073/pnas.96.11.5944>
- Mian L, Marwat GN, Latif A, Shah GS (1986). Testing and evaluation of various pesticides and cultivars on musk melon against melon fruit fly *Dacus cucurbitae* Coq. (Diptera: Tephritidae) in D.I. Khan. *Sarhad. J. Agric.* 2(2):403-410.
- Podoler H, Mazor M (1981). *Dirhinus giffardii* Silvestri (Hymenoptera: Chalcididae) as a parasite of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Some biological studies. *Acta Oecol.* 2:255-265.
- Sangvorn K, Sriplang K, Brockelman YW, Baimai V (2004). Laboratory evaluation of density relationships of the parasitoid, *Spalangia endius* (Hymenoptera: Pteromalidae), with two species of tephritid fruit fly pupal hosts in Thailand. *Science Asia* 30:391-397. <http://dx.doi.org/10.2306/scienceasia1513-1874.2004.30.391>
- Van D, Hoddle RM, Center T (2008). *Book on Control of Pests and Weeds by Natural Enemies*, Hoboken, NJ, USA: Wiley-Blackwell.
- Van Emden HF, Service MW (2004). *Book on pest and vector control*. Cambridge, UK: Cambridge University Press. <http://dx.doi.org/10.1017/CBO9780511616334>
- Van Lenteren JC, Bale JS, Bigler F, Hokkanen HMT, Loomans AJM (2006). Assessing risks of releasing exotic biological control agents of arthropod pests. *Annu. Rev. Entomol.* 51:609-634. <http://dx.doi.org/10.1146/annurev.ento.51.110104.151129> PMID:16332225
- Van SRA, Toscano NC, Bollmer GR, Kido K, Reynolds HT (1975). Increase of *Heliothis* spp. in cotton under various insecticide treatment regimes. *Environ. Entomol.* 4:993-996.
- Wang XG, Messing RH (2004). Potential interactions between pupal and egg or larval-pupal parasitoids of tephritid fruit flies. *Environ. Entomol.* 33(5):1313-13. <http://dx.doi.org/10.1603/0046-225X-33.5.1313>

Review

White rot (*Sclerotium cepivorum* Berk) - an aggressive pest of onion and garlic in Ethiopia: An overview

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Allium crops are the most indispensable vegetable crops used as condiments in most Ethiopian cuisine. Among them, onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) rightly called as “queen of kitchen”, belong to the family Alliaceae and considered as one of the most important vegetable and spice crops produced on large scale in Ethiopia, cultivated during dry and rainy seasons. The global pest, white rot, *Sclerotium cepivorum* Berk is one of the serious fungal disease reducing potential yields of onion and garlic crops in Ethiopia to a considerable degree. The development of this serious disease as depends on the environment, time and host plant, biological control; chemical and cultural practices, which are important in management of onion and garlic white rot disease. Therefore, in this paper attempts are made to collate information on pathogenicity of white rot to these two crops recorded in Ethiopia, their distribution, economic importance, damage and management options scattered over published and unpublished sources and avail them for use by researchers and development workers on white rot problem in the country. This paper is also believed to identify research gaps that need to be addressed.

Key words: *Allium cepa*, *Allium sativum*, Ethiopia, *Sclerotium cepivorum*.

INTRODUCTION

Allium crops are the most indispensable vegetable crops used as condiments in most Ethiopian cuisine. Among them, onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) rightly called as “queen of kitchen”, belongs to the family Alliaceae, and considered as one of the most important vegetable and spice crops produced on large scale in Ethiopia, cultivated during dry and rainy seasons (Rubatzky and Yamaguchi, 1997). Onion and garlic played an important dietary, as well as medicinal role for centuries. Onion is used in preparation of different foods,

and in therapeutic medicine in the country. Besides, it is rich in flavonoids like quercetin and sulfur compounds, such as allylpropylsulfide, that have been perceived benefits to human health (Griffiths et al., 2002) and used as a possible cancer preventive (Krest and Keusgen, 1999). Garlic is used in the treatment of headache, bites, worms, tumors and also could be reduced risk of cancer and chest pain in Ethiopia (Giday et al., 2003; Keusgen, 2002; Fekadu and Dandena, 2006). Han et al. (1995) reported that garlic has antibiotic properties and used to

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treat wound. It can inhibit and kill the bacteria, fungi, parasites and also lower blood pressure, blood cholesterol and blood sugar, prevent blood clotting, protect the liver and contain antitumor properties (Sovova and Sova, 2004). In Ethiopia, vegetable crops holders living nearer to urban centers largely practices vegetable farming, hence, the small volume of production recorded as well evidenced by the survey results of Central Statistics Authority of Ethiopia. The production of cash crops like garlic, onion and other spices is proved to be income generating for farmers, especially for those who have cultivated in limited lands or small holder farmers (FAO, 2006). As to production of these two vegetable crops contribute 2.78% to all crops production total, conversely, of the total production of vegetables, the same crops have the lion share, that is, about 31.69 and 42.76%, in that order (CSA, 2012). These two crops is produced mainly by subsistence farmers in the mid and lowlands of the country. The best growing altitude for onion and garlic under Ethiopian condition is between 700 and 1800 masl (Getechew and Asfaw, 2000).

Onion and garlic occupy an economically important place among vegetables in the country. A total of 30,478.35 ha of land was under Onion in the country, taking up about 15.25% land area covered by all vegetable crops at country level and yielding about 328,157.42 tons of produce by the peasant holders, contributing about 19.64% to the total country level all vegetable crops production. But, now-a-days the area under the production of onion is increased by far and productivity also increased up to 80 to 120 kg/ha under optimum condition. Garlic also taking up a total of 13,278.55 ha of land was under garlic in the country, taking up about 6.64% land area covered by all vegetable crops at country level and yielding about 123,961.46 tons of produce by the peasant holders, contributing about 7.42% to the total country level all vegetable crops production. Onion is extremely important vegetable crop not only for internal consumption but also as highest foreign exchange earner among the fruits and vegetables. However, the productivity of onion and garlic is affected by many biotic and abiotic stresses accounted for the low yield of onion and garlic in Ethiopia resulting in the use of low quality seeds, imbalanced fertilizers, uneven irrigations and less storability of the crop, lack of improved variety, lack of proper disease control and insect pest management practices, improved planting material, inappropriate agronomic practices in traditional production system and marketing facilities which are the prominent ones.

Globally, there are 66 diseases attacked *Allium* crops. Among them, white rot disease caused by *Sclerotium cepivorum* Berk is one of the most destructive disease causing heavy losses in onion and garlic (Coley-Smith, 1987; Crowe et al., 1980). In Ethiopia, the main limiting factor for onion and garlic production is the white rot disease caused by breaking of floral stalks, and thus, the

bulb yield and seed production is significantly reduced. The pathogen produces a great number of poppy seed-sized sclerotia, which can survive in soil for many years. Once established in a field, it permanently renders the field unusable for garlic and onion productions in Ethiopia for up to 40 or more years (Bo Ming et al., 2010). The pathogen is particularly difficult to manage, as it can remain dormant in the soil for many years until the next crop is planted. Thus, white rot disease is considered to be a major threat to the long-term viability of onion and garlic productions in Ethiopia. However, even at substantially reduced levels, onion and garlic grown in white rot -infested fields can suffer plant losses of up to 20 to 40% (Fullerton and Stewart, 1991), making avoidance or tolerance in these fields a cheaper ideal option. The development of this serious disease as depends on the environment, time and host plant, biological control; chemical and cultural practices, which are the important in management of onion and garlic white rot disease. Therefore, in this paper attempts are made to collate information on pathogenic of white rot of these two bulbous crops recorded in Ethiopia and other countries, their distribution, economic importance, ecology and epidemiology, damage and management options scattered over published and unpublished sources and avail them for use by researchers and development workers on white rot problem in the country reviewed. This paper is also believed to identify research gaps that need to be addressed.

ONION AND GARLIC PRODUCTION AND PRODUCTION CONSTRAINTS IN ETHIOPIA

Agriculture is the backbone of economy of Ethiopia. Onion and garlic is one of the most important vegetable and spice crops in Ethiopia and mainly produced as a cash sources for the farmers. Many people's also make their living by transporting and marketing these crops. In addition, the country earns foreign currency by exporting onion flowers. The plant has a very short growing period of only three to four months, which allows it to be grown between other crops during the short rains in the dry season. The plant is grown in most areas from onset of June to the end of September or October with rain fed and from November to March or from January to May with irrigation. Even though farmer's different areas name it differently, red to grayish red onion and garlic with medium to large size is commonly practiced in the country. However, planting materials used by farmers are usually heterogeneous in size, shape, color, pungency, storability and resistance to diseases. They are also differing in their time to reach maturity.

A variety of diseases and disorders affect onion and garlic related crops in Ethiopia both at field and in the storage. Accurate disease diagnosis is an important part of an integrated disease management (IDM) program that

assists identification of onion and garlic diseases and disorders that occur in the field and sometimes in storage. But there is a shortage of information and disease diagnosis, monitoring and management guidelines in the country. It is important that onion and garlic diseases be recognized early in their development so that effective management strategies can be implemented. Careful and regular monitoring of the crop can provide this timely information. Knowledge of the field and cropping history can also provide valuable clues to the potential for diseases or disorders, because some diseases occur annually for example, onion and garlic white rot whereas others are more incidental and unpredictable.

WHITE ROT INFECTION ON ONION AND GARLIC AND THEIR SYMPTOMS

Development of sclerotia germinating stimulants is underway and may lead to their introduction into commercial garlic production. Pathogenic activity of white rot increases as the root systems develop. Mycelial growth spreads upwards from the roots to the stem plate, the bulb, and then onto the leaves above ground. Soil conditions and the pathogen population in the field will determine the extent of damage inflicted on the onion and garlic crops. In cool moist soil where disease inoculum is high in the field, the sclerotia (survival structures) will germinate in the presence of onion and garlic root and bulb exudates. The fungus then attacks the roots, developing bulb and lower plant stem. Depending on soil conditions and inoculum levels, plants may be partially or completely destroyed. In the process, new sclerotia are formed that can persist in a dormant state in the soil for many years, waiting for a susceptible host. Under conditions that are less favorable to rapid sclerotial germination, the plant may survive to harvest, although the bulb may then rot in storage as disease symptoms continue to progress.

Normally, disease symptoms appear from mid-season to harvest. The white rot problem cannot be understated. This disease attacks the crop at all the stages of growth in the field, the leaves of both partly and fully developed plants turn yellow and wilt. There are also snow white mycelia on the bulb surface or at the crown of seedlings. The small rounded shape sclerotium also develops on the mycelia. The root system of the crops is destroyed completely and the crop leaves also turn yellow and wilt. The disease is more destructive during the early spring and the autumn seasons in Ethiopia. Evidence of this dastardly disease shows up when the leaves turn yellow and die back. Plants will sometimes keel over as the roots rot. A few plants may be affected at first, but this disease often spreads to infect whole rows of plants. Upon lifting affected plants, white fluffy fungal growth, a bit like cotton wool, can be seen around the bulb with tiny black globules, like poppy seeds among the fungus.

These black globules are resting bodies or sclerotia of the white rot fungus.

DISTRIBUTION OF WHITE ROT

Fedis in Harerge; Huruta, Sire, Shirka, Bokoji, and Arsi Negelle in Arsi; Ambo, Wolliso, Godino, Kesem, and Majete in Shewa; Bure, and the vicinities of Dibre Markos in Gojam; and Warailu and Warababo in Wello are the most onion and garlic growing areas of the country (Getachew and Asfaw, 2000). In South, South-West and North-East West Showa Zone, Ambo high lands, garlic is widely cultivated using rain supplemented by irrigation. *Sclerotium cepivorum* has caused great damage in diverse regions of Ethiopia (Tamire et al., 2007). Furthermore, *S. cepivorum* is an issue of great importance for dry climate producers in Ethiopia (Zewide et al., 2007). Zeray and Yesuf (2013) reported that the distribution, incidence and prevalence of garlic white rot in major growing districts of South-East and East Tigray in Ethiopia. Ararsa and Thangavel (2013) reported the incidence, prevalence and severity of the white rot in Ambo and Toke Kutaye districts of Western Showa, Ethiopia. Tamire et al. (2007) reported white rot incidence ranging from 37.28 to 42% in cultivated fields of garlic in Northern Showa of Ethiopia. Onion and garlic white rot caused by *S. cepivorum* is a major production threat of onion and garlic in Tigray Regional State of the country (Zeray, 2011). During favorable weather conditions, and when susceptible varieties are in the production system the disease can cause 100% yield loss (Zeray, 2011). It is prevalent in many onion and garlic growing regions worldwide, but its intensity can vary with different cultural practices and environmental factors.

ECOLOGY AND EPIDEMIOLOGY OF WHITE ROT

The sclerotia that form on the decaying host will lay dormant until a host plant's root exudates stimulate germination specifically root exudates that are unique to *Allium* spp. Cool weather is also needed for germination of sclerotia and hyphal growth. The soil moisture levels optimal for host root growth are also optimal for sclerotia germination. Mycelium will grow through the soil, and once it encounters a host root the fungus will form appresoria, structures whose purpose is to aid in the attachment and penetration of the host. Mycelium can grow outwards from the roots of one plant to the roots of a neighboring plant, and it is by this method that the pathogen can move down a planted row. Sclerotia are formed on the decaying host tissue, and once the host tissue completely decays the sclerotia are free in soil. If the bulbs survive long enough to be placed into storage, the pathogen may continue to decay the bulbs if there is high humidity and low temperatures. If the bulbs are stored dry then the disease may not spread

but bulbs infected in the field will continue to decay (Crowe, 2008; Ararsa and Thangavel, 2013).

White rot originates from small hard-walled storage structures, called sclerotia, which are produced in abundance in infected plant tissue, and are 200 to 500 μm in diameter (Maude, 2006). Sclerotia rely on these volatiles to germinate, environmental conditions also influence germination, with cool weather (14 to 18°C) and moderate-to-high soil moisture favoring germination and infection (Maude, 2006), though some research has suggested sclerotial germination is fairly independent of soil moisture content. Though, each sclerotium can only grow hyphae 1 to 2 cm in length (Maude, 2006), the high density at which onions and garlic are typically planted makes infection from infested soil essentially inevitable. Once contact is made with an *Allium* plant root, the hyphae forms an appressorium, a swollen hyphal tip that presses onto the root epidermis, from whence first a fungal toxin, oxalic acid, and then degradative fungal enzymes, endopolygalacturonases and pectinases, are produced. Oxalic acid degrades tissue in concert with the degradative enzymes that follow its secretion, leading to cell death directly beneath the appressorium. The appressorium then produces an infection plug, which penetrates between the cell wall junctions and deep into the root (Maude, 2006). The advancing penetrative hyphae branch out and secrete oxalic acid ahead of the hyphal tips, followed by endopolygalacturonase and pectinase enzymes. This acid and enzyme system continues to degrade tissue allowing the pathogen to penetrate further and absorb the nutrients released from the *Allium* tissue.

The pathogen rapidly colonizes the root system and base plate, then infects the bulb tissue and extends mycelia outwards to infect the roots of neighboring onion and garlic plants. Once the bulb is infected the plant soon loses vigor, the leaves yellow and wilt, and fluffy, white mycelia fill the bulb and then produce abundant sclerotia to precipitate future infections (Maude, 2006; Mueller et al., 2006). The hard-walled sclerotia formed during infection are incredibly tough, and can remain dormant yet viable in the soil for many years (Maude, 2006), some evidence suggesting up to two decades (Mueller et al., 2006), meaning that once a field is infested with *S. cepivorum*, *Allium* cultivation may not be possible there for many years.

Enough sclerotia from an initially high population may survive 20 to 30 years or more in soil without the presence of an *Allium* host to incite unacceptable levels of disease in the next *Allium* crop. Only *Allium* spp. such as onion, leek, and shallot are attacked. Sclerotia can infect plants from 12 inches below the surface. One sclerotium can infect a group of 20 to 30 adjacent plants. Fungal activity is favored by cool soils and is restricted above 75°F. Once the disease is in a field, it is very difficult to grow *Allium* spp. successfully. Leaves decay at the base, turn yellow, wilt, and topple over. Older leaves

collapse first. Roots are rotted, and the top of the plant can be pulled out of the ground easily. Fluffy white mycelium may be on remaining roots and bulb. The affected bulb may become watery, and the outer scales crack as it dries and shrinks. Small (0.02 inch) sclerotia form in affected bulb parts and on the surface, often around the neck. Sclerotia are smaller and rounder than those of the neck rot disease.

Onion and garlic may be attacked at all stages of crop growth. Early attacks cause poor crop establishment; later infections produce yellowing and wilting, which in some cases results in the complete collapse of the plant. Affected plants have a grey-white fluffy mycelium on their stem bases, which gives the disease its name: white rot. The disease is caused by the sclerotia of *Sclerotium cepivorum*, which perennate in the soil. Sclerotia germinate producing hyphae that infect the roots and stem plates of onions. New sclerotia are formed in the stem base tissues of onions and these are released into the soil when plants die or are harvested (Entwistle and Munasinghe, 1978).

Host and soil effects are important in the epidemiology of white rot. In the first time, for plant infection to occur, the sclerotia held in check by soil fungistasis have to receive a stimulus from specific factors released as exudates by the roots of onions (Esler and Coley-Smith, 1983). When this occurs sclerotia germinate producing hyphae that may grow 1 to 2 cm through the soil to infect the roots of onions. They penetrate the root epidermis producing appressoria and infection cushions and the hyphae enter between cell wall junctions (Metcalf and Wilson, 1999). Then they invade the hypodermis and grow into the cortex of the roots. During the early stages of infection, cell death is limited to the cells penetrated by the hyphae of *S. cepivorum*; however, onion cell walls in the path of the leading infection hyphae often dissolve before the hyphal tips reach them. This was shown to be indicative of enzymic action by certain pectinases (Metcalf and Wilson, 1999). Plant-to-plant spread of infection may occur in densely-sown or closely-spaced crops.

Secondly, soil temperature is a critical factor affecting germination of sclerotia, mycelial growth and root infection of onions. Sclerotial germination is favoured by temperatures between 9 to 20°C and the disease development on onion and garlic by white rot is ranged from 6 to 24°C; outside this range, germination is slow but returns to normal when temperature is restored to 15°C (Crowe and Hall, 1980). Thus, in Ethiopia, sclerotial germination is poor during the winter but improves in spring and early summer; later in the summer, germination may be inhibited by high soil temperatures-sclerotia cannot survive temperatures of 35°C and above (Tamire et al., 2007). As a result, disease losses are high in overwintered crops in the autumn and spring, when temperatures are suitable for infection, but white rot may be of minor importance in hot summers.

Sclerotial germination is little affected by pH values varying from 4.8 to 8.5 (Entwistle and Granger, 1977); there was a decrease in germination of sclerotia as soils became drier or wetter than field capacity (-300 millibars) (Crowe and Hall, 1980). Clarkson et al. (2004) showed that over 90% of sclerotia were degraded at high water potentials where the soil was nearly saturated. This confirmed the findings of previous workers (Crowe and Hall, 1980) and those of Leggett and Rahe (1985) that used flooding to degrade sclerotia as a means of controlling the disease. Sclerotia may persist in the soil from at least 4 to 20 years (Entwistle, 1990). Attempts to forecast white rot based on assessments of the numbers of sclerotia per given weight of soil have met with variable results within and between countries (Entwistle, 1990). Newer models based on inoculum dynamics (Backhouse, 2003) have so far failed because of insufficient data to develop the relationship for the production of new sclerotia based on the size of infected bulbs. The maximum increase in numbers of sclerotia in the soil occurs at the end of the onion growing season and this will affect the following crop but only if it is an onion crop.

MANAGEMENT OF WHITE ROT

A number of chemical, biological, physical and cultural control methods have been devised or employed, as well as integrative control systems that combine methods for maximum effectiveness. On top of this, a great deal of effort has gone into the search for onion and garlic's with genetic resistance to white rot. Management of diseases caused by soil borne pathogens especially those that produce sclerotia is very difficult and need a multi-pronged management strategy. Crop rotation used for primary inoculum reduction (Banks and Edgington, 1989) but has been viewed as impractical for *Allium* white rot control due to the persistence and longevity of the sclerotia in the soil, soil solarization (Prados-Ligero et al., 2002), biological control agents (Gerlagh et al., 1996), sclerotia germination stimulants (Tyson et al., 2000) and composted onion waste (Coventry et al., 2002), host resistant, the difference in susceptibility exist among *Allium* species probably based on differences in concentration and composition of precursor to stimulatory volatiles contained in the host tissue (Brix and Zinkernagel, 1992). The management of white rot is a priority for the onion and garlic industries in Ethiopia, currently being undertaken to investigate suitable management options at both pre-planting and post-planting stages.

In Ethiopia, research effort on host resistant against onion and garlic white rot is very limited. The management methods of the diseases of onion and garlic are followed as mainly crop rotation, avoidance and sanitation to proper storage methods (Banks and Edgington, 1989). Conventional methods of control of

diseases of onion include the use of chemicals, organic means, and through integrated management efforts. As a result, attempts to manage the disease have focused on reducing the populations of sclerotia in the soil through biological control. Research on biological control of plant pathogens has received much attention in recent years, as a means of increasing crop production by avoiding a number of problems related to chemical control, and hence, developing practices compatible with sustainable agriculture (Arasa and Thangavel, 2013).

CULTURAL AND PHYSICAL CONTROL OF WHITE ROT

One of the most important forms of white rot control is simple hygiene or sanitation. It can be achieved through cleaning equipment carefully between fields and where possible leaving equipment used in white rot infested fields for use only in those fields, farmers can avoid spreading the sclerotia which introduce the disease to uncontaminated growing areas (Entwistle, 1992). Beyond simple hygiene, there are some control methods that aim to reduce the inoculum density of *S. cepivorum* sclerotia in infested soil, so as to slowly reduce the severity of disease in those fields. One of these methods is soil solarization, in which plastic sheeting; usually polyethylene is spread over or shredded into the soil to concentrate sunlight, raising the soil temperature to 36 to 50°C (Katan, 1987) during part of the day. Successive cycles of such heating throughout the hotter months, with soil temperatures above 35°C, the maximum temperature *S. cepivorum* can survive, gradually kill off most sclerotia in the soil (Katan, 1987). This method is particularly effective in hot, dry, sunny climates such as the Middle East (Satour et al., 1989), Spain (Meleró-Vara et al., 2000), and parts of Australia (Porter and Merriman, 1983), far less so in areas with high soil moisture content and less sun, such as temperate New Zealand (Stewart and McLean, 2007). In cases where the climate is appropriate, some fields have seen reductions in inoculum density of near 100% (Porter and Merriman, 1983; Satour et al., 1989), while results in wetter, cooler countries have been far more subdued (Brewster, 2008). Because the technique requires 3 to 4 months in which no other crops can be grown, as well as the expense of coating infested fields with plastic, soil solarization might only be cost-effective in countries where the climate aids the process. Many researchers have identified *S. cepivorum* sclerotia as sensitive to high soil moisture (Alexander and Stewart, 1994; Clarkson et al., 2004), as this causes cracks and weaknesses in the sclerotial rind. *S. cepivorum* sclerotia rely on moderate soil moisture to germinate.

Another form of physical control by which sclerotial density can be decreased is the use of germination stimulants and soil amendments. These amendments are artificial chemicals or organic mulches that stimulate *Sm*.

Cepivorum sclerotia to germinate by mimicking or containing the volatile chemicals in *Allium* root exudates.

The most common artificial amendment is diallyldisulphide (Stewart and McLean, 2007), a synthetic form of the volatile that stimulates sclerotial germination. If sclerotia germinate in the absence of a viable host they run out of stored nutrients and die, reducing the inoculum density of infested fields (Brewster, 2008). Similar results can be achieved using onion or garlic oil or waste compost produced in onion processing plants as an organic germination stimulant (Brewster, 2008). The application of artificial amendments or organic amendments can make a field with an otherwise prohibitive level of *S. cepivorum* sclerotia more viable for use in *Allium* cultivation. One advantage is that non-*Allium* crops can be grown during the period of amendment (Stewart and McLean, 2007).

Some work has also suggested that *Brassica* residue incorporation or intercropping may have a suppressive effect on *S. cepivorum* sclerotial germination (Ulacio-Osorio et al., 2006; Zewide et al., 2007), though this avenue has not been fully investigated yet. Soil amendment treatments, as with soil solarization, have a cost associated with application which limits its use, particularly in treating infestations that cover larger areas. As part of an integrated control program, however, germination stimulants may play an important role in protecting *Allium* cultivation from the legacy of white rot infestations.

BIOLOGICAL CONTROL OF WHITE ROT

Biological control of plant pathogens is currently accepted as the key practice in sustainable agriculture because it is based on the management of natural resources, that is, certain rhizosphere organisms that are common components of ecosystem, known to develop antagonistic activities against harmful organisms. Among microorganisms, fungi are the potential candidate for the biological control of plant pathogens and several antagonistic fungi viz., *Trichoderma*, *Gliocladium*, *Talaromyces* and Arbuscular Mycorrhizal Fungi (AMF) have shown enormous potential as bio-control agents against different soil borne and foliar diseases (Lo et al., 1996). In Egypt, research has identified *Trichoderma harzianum* as a potential biological control agent for use against white rot, providing 86% reduction in disease in one test (Abd-El-Moity, 1992). This is reinforced by work in Mexico, identifying the C4 strain of *T. harzianum* as providing protection against white rot in garlic (Miranda et al., 2006). Tasmanian researchers have achieved promising 91.2% disease suppression with *Trichoderma* isolate Td22 in one study (Metcalf and Wilson, 2001). In New Zealand, under controlled conditions, *Chaetomium globosum* and *Trichoderma* isolate C62 provided an average of about 73% suppression over two years,

though attempts to modify the agents for use in seed coats or pellets for dispersal reduced these figures to around 50%, similar to the control provided by many fungicides (Kay and Stewart, 1994).

Similar work in the United Kingdom has identified two strains of *Trichoderma viride* that provided significant protection against white rot, at levels comparable to tebuconazole (Clarkson et al., 2002). In the same country, other workers found potential in *Coniothyrium minitans* for control of white rot equal to that provided by calomel, without the phyto- and environmental toxicity associated with that chemical's use (Ahmed and Tribe, 1977). Research in Canada has also suggested *Bacillus subtilis* and *Penicillium nigricans* may provide protection from white rot up to the level shown by industry standard fungicides like iprodione and vinclozolin (Utkhede and Rahe, 1980). Clarkson et al. (2004) showed that isolates of *T. viride* effectively degraded sclerotia of *S. cepivorum* (four isolates) in four onion soil types (silty clay, sand, silt and peat) under a range of temperature and soil moisture conditions.

In Ethiopia, Ararsa and Thangavel (2013) reported that the native strains of AMF and *Trichoderma* species for plant growth promotion and biological management against white rot caused by *S. cepivorum* in onion at West shewa, Ethiopia. Five strains with 20 isolates of *Trichoderma* and 10 species of AMF were isolated from cultivated field of onion rhizosphere soils and screened for their bio-control potential against white rot pathogen. Among ten AMF species, *Glomus aggregatum* (Awaro isolate) was selected for using as a bio-control agent. The bio-control potential of bio-agents against white rot pathogen has been studied by the effect of *G. aggregatum* alone or in combination with *Trichoderma* spp. Dual inoculation with both *G. aggregatum* and *S. cepivorum* was almost comparable with control. Incidence of *S. cepivorum* was significantly reduced in bulbs and greater plant growth was observed in plants related with inoculation of both *G. aggregatum* and *T. harzianum* (ATH1). Plants inoculated with *S. cepivorum* alone showed pronounced disease symptoms with mean disease incidence of 90%. Treatments with combined inoculation of *G. gregatum* and *T. harzianum* (ATH1 isolate) significantly reduced the white rot of onion. The overall reduction in the incidence of white rot was 56.19% for *G. aggregatum* with *T. harzianum* and 56.22% for treatments with *T. harzianum*. Ararsa and Thangavel (2013) reported that the bio-control potential of seven indigenous *Trichoderma* species viz., *T. asperillum*, *T. atroviride*, *T. hamatum*, *T. harzianum*, *T. longibrachiatum*, *T. oblongisporum* and *T. viride* were evaluated by *in-vitro* and *in vivo* for their antagonistic and inhibition potential against garlic white rot disease caused by *S. cepivorum*. The overall inhibition effect of the antagonists on the pathogen's colony growth ranged from 43.9 to 59.3%. Isolate of *T. hamatum* was the highest inhibition effect (59.3%) followed by *T. harzianum* (53.3%), *T. oblongisporum*

(52.7%), *T. viride* (51.8%), *T. asperillum* (50.2%), *T. longibrachiatum* (47.2%) and *T. atroviride* (43.9%) which were not significantly different from each other. Incidence of *S. cepivorum* was significantly reduced in bulbs of garlic and also improved plant growth was observed in plants inoculated with single inoculation of *T. hamatum* alone followed by *T. harzianum* alone and in combined inoculation of *T. hamatum*, *T. harzianum*, *T. hamatum* and *T. oblongisporum* isolates. Plants inoculated with *S. cepivorum* alone showed pronounced disease symptoms with mean disease incidence of 90.5%. Treatments with combined inoculation of *T. hamatum* and *T. harzianum* isolates significantly reduced (62.22%) the white rot of garlic followed by the combined inoculation of *T. oblongisporum* and *T. harzianum* and *T. hamatum* and *T. viride*. The overall reduction in the incidence of white rot was 62.22% in the treatment of *T. hamatum* and *T. harzianum* isolates with pathogen followed by 53.72% for *T. oblongisporum* and *T. harzianum* with pathogen.

HOST RESISTANCE AND BREEDING

It was noted that resistance to white rot in onions and other *Allium* species can be correlated with the amount of volatile precursor root exudates the plant produces (Brix and Zinkernagel, 1992). *Allium* species and cultivars with lower volatile precursor levels, and those that produce mainly non-stimulatory exudates like methylcysteine sulphoxides, stimulate less germination (Esler and Coley-Smith, 1983). This may indicate that breeding for low volatile precursor-producing varieties would reduce disease incidence, but research suggests that the levels of allylcysteinesulphoxides required to stimulate germination are fairly low (Esler and Coley-Smith, 1983). In addition to these concerns, a number of variable environmental factors such as temperature and moisture levels influence stimulatory root exudates levels, making any objective comparisons between cultivars difficult to make (Brix and Zinkernagel, 1992). Brix and Zinkernagel (1992) also revealed that the size of the root mass affects disease incidence, first by affecting the likelihood of roots being close to sclerotia and secondly by affecting the spread of disease between plants. This means that *Allium* species with smaller and less spread-out root masses may incur less disease, but this characteristic is not correlated with other commercially useful traits, and is also heavily influenced by environmental conditions like water availability (Brix and Zinkernagel, 1992). With this in mind, breeding for small root masses would be unlikely to yield consistently resistant plants, and would take a prohibitively long time to achieve.

The influence of environmental variation on variation in disease incidence has plagued attempts at identifying resistance in *Allium* to white rot infection (Utkhede et al., 1982). Because the expected differences in susceptibility

between cultivars is so small, introducing even mild variation from outside makes analyzing results very difficult. While the use of carefully controlled laboratory evaluations may avoid this variation, it also introduces the problem of attempting to apply laboratory conclusions to field conditions. Without extensive evaluations for resistance under controlled conditions, and comparisons to field assessments under typical cultivation conditions, the search for resistant germplasm is flawed, and with these caveats met, it remains an expensive and time consuming process (Hunger et al., 2002). Even some of the most successful work in identifying resistance phenotypes has been painstakingly slow (Nabulsi et al., 2001), and still holds no guarantee of success. As Brix and Zinkernagel (1992) put it, "no [*Allium*] species is known which shows a consistently high degree of resistance to *S. cepivorum*."

CHEMICAL CONTROL OF WHITE ROT

Over the last 30 to 40 years chemical control of onion and garlic white rot has largely depended on the use of dicarboximide fungicides such as procymidone, iprodione and vinclozolin which can be used as seed dressings and foliar sprays. The first of these is particularly effective at reducing the severity of disease and loss of yield when growing onions or garlic in *S. cepivorum* infested fields (Stewart and Fullerton, 1991). The use of such fungicides has been important because it allows fields otherwise unusable for *Allium* cultivation to return to productive use. In addition to limited effectiveness, the phenomenon of enhanced degradation of dicarboximide fungicides by resident soil micro-organisms has been detected in fields where such fungicides have been used regularly, with some research identifying a 90% loss of dicarboximide fungicides in soils pre-treated with them within 7 days (Garcia-Cazorla and Xirau-Vayreda, 2005). With the number of sites where onions and garlic can be readily grown so small, and the proportion of those sites infested with white rot increasing, the gradual selection of soil microbes capable of enhanced degradation of dicarboximide fungicides in those soils (Athiel et al., 1995) makes the use of such fungicides ultimately a finite solution (Clarkson et al., 2002). An alternative fungicide, tebuconazole, has offered hope in the control of white rot in infested fields, showing better control than the best dicarboximide fungicide procymidone in some comparisons (Duff et al., 2001). However, tebuconazole is best suited for foliar spraying, showing phytotoxicity when applied as a seed dressing (Fullerton et al., 1995), though granular applications may avoid this (Fullerton et al., 1995). Tebuconazole and triadimenol, both triazole fungicides, may well hold the most promise for ongoing control of white rot (Tyson et al., 1999; Clarkson et al., 2002), with no evidence yet arising to suggest that enhanced degradation of these fungicides occurs in the

field (Pung et al., 2007). However, there is no reason to suppose that soil microbes in treated areas will not eventually develop the ability to degrade these fungicides at an enhanced rate, particularly if farmers become more reliant on their use, thereby selecting more heavily for soil microbes that can degrade them.

While the use of fungicides can reduce disease severity in infested fields to a point where cultivation is economically worthwhile, regular *Allium* cultivation in such fields increases sclerotial density over time, increasing disease severity in subsequent seasons (Zewide et al., 2007a). One solution to this problem is the chemical eradication of the fungal propagules, sclerotia, by fumigation with methyl bromide. By reducing the inoculum density in white rot infested fields, farmers can decrease the risk associated with *Allium* cultivation. However, there is not an absolute correlation between inoculum density and disease severity (Melero-Vara et al., 2000), and even a relatively small persisting inoculum level can result in economically significant levels of disease. Methyl bromide fumigation, while fairly effective at reducing sclerotial numbers in the field, is environmentally damaging, and is being phased out in the developed world (Avila-Miranda et al., 2006).

In addition to the above concerns, most fungicides display some kind of toxicity, and those used to control white rot are no exception. Thiram and the sclerotial germination stimulant diallyldisulphide (DADS) are both designated 'very toxic to humans' in the New Zealand Agrichemical Manual (2005). Procymidone, one of the most traditionally reliable fungicides used in white rot control is a suspected reproductive/developmental toxin, as are next generation fungicide triadimenol and soil sterilant methyl bromide. Vinclozolin, a dicarboximide fungicide like procymidone and iprodione, has been shown to cause cross-generational tumours and abnormalities in laboratory mice (Anway et al., 2006). Mercury chloride, better known as the fungicide calomel, is still used for white rot control in developing countries (Miranda et al., 2006), despite the World Health Organization (WHO) classifying it as 'extremely hazardous,' the highest hazard rating they assign (WHO, 2004).

Fungicides are among the most effective options for onion and garlic white rot disease management in Ethiopia. According to Tamire et al. (2007) reported that the systemic as well as non-systemic fungicides significantly reduced incidence of white rot, its progress rate, severity, and there by improved garlic yield. Fullerton and Stewart (1991) also found that Rocymidon reduced incidences of white rot up to 75 to 95% applied as bulb and soil treatment. According to Melero-Vara et al. (2000) and Duff et al. (2001) reported that the tebuconazole was effective in reducing the incidence and progress of the disease and in increasing the yield when applied as a clove treatment.

The control of onion and garlic white rot disease has

been almost exclusively based on the application of chemical fungicides. Several effective fungicides have been recommended for use against this pathogen, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, fungicide reused and other health and environmental hazards. In attempt to modify this condition, some alternative methods of control have been adopted. Recent efforts have focused on developing environmentally safe, long lasting and effective bio-control methods for the management of plant diseases.

INTEGRATED DISEASE MANAGEMENT OF WHITE ROT

The most effective control systems to date have involved the integration of a number of systems for managing white rot. Clarkson et al. (2004) found that high soil moisture allowed their *Trichoderma* BCAs to better degrade sclerotia, as well as speeding up degradation generally, by weakening the sclerotial rind (Alexander and Stewart, 1994). Abd-El-Moity (1992) selected a benomyl-resistant strain of *T. harzianum* for his research, as a BCA tolerant to certain fungicides would be far more useful in modern *Allium* cultivation. Under an integrated control system cultural control methods slow the spread of the disease, while physical control methods like soil solarisation, flooding and germination stimulants are used to reduce the inoculum density in fields already infested with *S. cepivorum*. Then, if previously-infested fields are planted with *Allium* species, the use of fungicidal seed coats, dips and foliar sprays reduces the incidence of disease in those fields, while biological control agents work antagonistically against the sclerotia in the soil. The use of resistant cultivars could also provide another weapon in this arsenal, but no such cultivars have been developed to a commercial standard (Clarkson et al., 2002). The use of an *Allium* cultivar specifically and directly transformed to express a gene for white rot resistance might allow these other control methods to work more effectively, or even rule out the need for some of the more economically and environmentally expensive control methods, such as soil.

CONCLUSIONS

Whit rot disease is the most pressing problem to the subsistence onion and garlic farming community more than any other category of vegetable diseases in Ethiopia. The problem is particularly acute during moist rainy season. Great attention should be rendered to creating the necessary capacity in white rot fungal disease research to effectively deal with the white rot fungal disease problem in onion and garlic areas of Ethiopia. Furthermore, the white rot problem in the

country in general and the onion and garlic growing areas in particular is growing from bad to worse. Some pathological research in the country during the past, there is still a lot remains to be done in the future. Emphasis should be given to the wealth of indigenous knowledge and build on there to develop more viable technologies that are within reach the great majority of resource poor small-scale farmers in the country.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

- Abd-El-Moity TH (1992). The use of *Trichoderma* spp. to control soil-borne pathogens in Egypt. *Biological Control of Plant Diseases Progress and Challenges for the Future*, Edited by Tjamos, ES, Papavizas GC, Cook RJ Plenum Press, New York pp. 255-258.
- Ahmed AHM, Tribe HT (1977). Biological control of white rot of onion (*Sclerotium cepivorum*) by *Coniothyrium minitans*. *J. Plant Pathol.* 26:75-78. <http://dx.doi.org/10.1111/j.1365-3059.1977.tb01027.x>
- Alexander BJR, Stewart A (1994). Survival of sclerotia of *Sclerotinia* and *Sclerotium* spp in New Zealand horticultural soil. *Soil Biol. Biochem.* 26(10):1323-1329. [http://dx.doi.org/10.1016/0038-0717\(94\)90213-5](http://dx.doi.org/10.1016/0038-0717(94)90213-5)
- Anway MD, Leathers C, Skinner MK (2006). Endocrine disruptor Vinclozolin induced epigenetic transgenerational adult-onset disease. *Endocrinology* 147(12):5515-5523. <http://dx.doi.org/10.1210/en.2006-0640> PMID:16973726
- Ararsa L, Thangavel S (2013). Evaluation of Arbuscular Mycorrhizal Fungi and *Trichoderma* Species for the Control of Onion White Rot (*Sclerotium cepivorum* Berk) *J. Plant Pathol. Microbiol.* 4:159.
- Athiel P, Alfizar, Mercadier C, Vega D, Bastide J, Davet P, Brunel B, Cleyet-Marel JC (1995). Degradation of Iprodione by a soil *Arthrobacter*-like strain. *Appl. Environ. Microbiol.* 61(9):3216-3220. PMID:7574630 PMID:PMCid:PMC167600
- Avila-Miranda ME, Herrera-Estrella A, Pena Cabriales JJ (2006). Colonization of the rhizosphere, rhizoplane and endorhiza of garlic (*Allium sativum* L.) by strains of strains of *Trichoderma harzianum* and their capacity to control *Allium* white-rot under field conditions. *Soil Biol. Biochem.* 38:1823-1830. <http://dx.doi.org/10.1016/j.soilbio.2005.11.036>
- Backhouse D (2003). Modelling inoculum dynamics and epidemiology of onion white rot, in Eighth International Congress of Plant Pathology, (eds D. Swain and S. Zydenbos) Christchurch, New Zealand, February 2nd-7th P. 102.
- Banks E, Edgington LV (1989). Effect of integrated control practices on the Onion white rot pathogen in organic soil. *Candan J. Plant Pathol.* 11:268-272. <http://dx.doi.org/10.1080/07060668909501111>
- Bo Ming W, Mike D, Tom T (2010). Developing New Integrated Strategies for Controlling White Rot in Garlic.
- Brewster JL (2008). Onions and Other Vegetable Alliums, 2nd Ed. *Crop Prod. Sci. Hortic. Series P.* 15. <http://dx.doi.org/10.1079/9781845933999.0000>
- Brix HD, Zinkernagel V (1992). Screening for resistance of *Allium* species to *Sclerotium cepivorum* with special reference to non-stimulator resistance. *Plant Pathol.* 41:308-316. <http://dx.doi.org/10.1111/j.1365-3059.1992.tb02352.x>
- Clarkson JP, Mead A, Payne T, Whipps JM (2004). Effects of environmental factors and *Sclerotium cepivorum* isolate on sclerotial degradation and biological control of white rot by *Trichoderma*. *Plant Pathol.* 53:353-362. <http://dx.doi.org/10.1111/j.0032-0862.2004.01013.x>
- Clarkson JP, Payne T, Mead A, Whipps JM (2002). Selection of fungal biological control agents of *Sclerotium cepivorum* for control of white rot by sclerotial degradation in a UK soil. *Plant Pathol.* 51:735-745. <http://dx.doi.org/10.1046/j.1365-3059.2002.00787.x>
- Coley-Smith JR (1987). *Sclerotium cepivorum* Berk. in *European Handbook of Plant Diseases*, (eds I. M. Smith, J. Dunez D. H., Phillips R. A. Lelliot and S. A. Archer) Blackwell Scientific Publications, pp. 446-447.
- Coventry E, Noble R, Mead A, Whipps JM (2002). Control of white rot (*Sclerotium cepivorum*) with copoted garlic waste. *J. Soil Bio-chem.* 34:1037-1045. [http://dx.doi.org/10.1016/S0038-0717\(02\)00037-8](http://dx.doi.org/10.1016/S0038-0717(02)00037-8)
- Crowe FJ, Hall DH, Greathead AS, Baghott KG (1980). Inoculum density of *Sclerotium cepivorum* and the incidence of white rot of onion and garlic. *Phytopathology* 70:64-69. <http://dx.doi.org/10.1094/Phyto-70-64>
- Crowe FJ, Hall DH, Greathead AS, Baghott KG (1980). Inoculum Y density of *Sclerotium cepivorum* and the incidence of white rot of onion and garlic. *Phytopathology* 70:64-69. <http://dx.doi.org/10.1094/Phyto-70-64>
- Crowe FJ (2008). White Rot in compendium of onion and garlic diseases and pests. Schwartz, J. F. ed. APS Press, St. Paul, MN. pp. 22-26.
- Crowe FJ, Hall DH (1980). Soil temperature and moisture effects on sclerotium germination and infection of onion seedlings by *Sclerotium cepivorum*. *Phytopathology* 70:74-78. <http://dx.doi.org/10.1094/Phyto-70-74>
- CSA (Central Statistical Authority) (2012). Agricultural Sample Survey. Report on forecast of area and production of major crops, Statistical Bulletin, No. 506, Addis Ababa, Ethiopia.
- Duff AA, Jackson KJ, O'Donnell WE (2001). Tebuconazole (Folicur®) shows potential in the control of white rot (*Sclerotium cepivorum*) in garlic in subtropical queensland, Australia. *Proceedings of the 2nd International Symposium on Edible Alliaceae.* pp. 247-250.
- Entwistle AR (1990). Root diseases, in *Onions and Allied Crops: Volume 11 - Agronomy, Biotic Actions, Pathology, and Crop Protection*, (eds H. D. Rabinowitch and J. L. Brewster) CRC Press, Inc., Boca Raton, Florida, pp. 103-154.
- Entwistle AR (1992). Controlling *Allium* white rot (*Sclerotium cepivorum*) without chemicals. *J. Phytoparasitology.* 20:121-125. <http://dx.doi.org/10.1007/BF02980422>
- Entwistle AR, Granger J (1977). *Sclerotium* germination studies. Report of the National Vegetable Research Station for 1976, The British Society for the Promotion of Vegetable Research, Wellesbourne, Warwick, CV35 9EF, UK, pp. 97-98.
- Entwistle AR, Munasinghe HL (1978). Epidemiology and control of white rot disease of onions, in *Plant Disease Epidemiology*, (eds P. R. Scott and A. Bainbridge) Blackwells Scientific. PMID:PMC1608055
- Esler G, Coley-Smith JR (1983). Flavour and odour characteristics of species of *Allium* in relation to their capacity to stimulate germination of sclerotia of *Sclerotium cepivorum*. *Plant Pathol.* 32:13-22. <http://dx.doi.org/10.1111/j.1365-3059.1983.tb01296.x>
- FAO (Food and Agriculture Organization) (2006). Food security in Ethiopia, Agriculture and Consumer protection Department Fekadu, M., and G. Dandena. 2006. Review of the status of vegetable crops Production and marketing in Ethiopia. *Uganda J. Agric. Sci.* 12(2):26-30.
- Fekadu M, Dandena G (2006). Review of the status of vegetable crops production and marketing in Ethiopia. *Uganda J. Agric. Sci.* 12(2):26-30.
- Fullerton R, Stewart A (1991). Chemical control of onion white rot (*Sclerotium cepivorum* Berk) in the pukekohe district of New Zealand. *New Zealand J. Crop. Hort. Sci.* 19:121-127. <http://dx.doi.org/10.1080/01140671.1991.10421789>
- Fullerton RA, Stewart A, Slade EA (1995). Use of demethylation inhibiting fungicides (DMIs) for the control of onion white rot (*Sclerotium cepivorum* Berk.) in New Zealand. *New Zealand J. Crop. Hort. Sci.* 23:121-125. <http://dx.doi.org/10.1080/01140671.1995.9513878>
- Garcia-Cazorla J, Xirau-Vayreda M (2005). Cross enhanced degradation of dicarboximide fungicide residues in soils. *Am. J. Enol. Viticult.* 56(1):77-80.
- Gerlagh M, Whipps JM, Budge SP, Goossen-van De Geijn HM (1996). Efficacy of isolates of *Coniothyrium minitans* as mycoparasites of *Sclerotium cepivorum*, and *Botrytis cinerea* on tomato stem pieces.

- Eur. J. Plant Pathol. 102:787-793.
<http://dx.doi.org/10.1007/BF01877154>
- Getachew T, Asfaw Z (2000). Achievement in Shallot and Garlic Research. Ethiopia Agricultural Research Organization, Addis Abeba, Ethiopia.
- Giday M, Asfaw TZ, Elmquist, Woldu Z (2003). An Ethonobotanical study of medicinal plant used by the Zay people in Ethiopia. J. Ethonopharmacol. 85:43-52.[http://dx.doi.org/10.1016/S0378-8741\(02\)00359-8](http://dx.doi.org/10.1016/S0378-8741(02)00359-8)
- Griffiths G, Trueman L, Crowther T, Thomas B (2002). Onions: A global benefit to health. *Phytother. Res.* 17:603-615.
<http://dx.doi.org/10.1002/ptr.1222> PMID:12410539
- Han L, Lawson G, Han Han P (1995). A spectrophotometric method for quantitative determination of allicin and total garlic thiosulfates. J. Ana. Chem. 225:157-160.
- Hunger SA, McLean KL, Eady CC, Stewart A (2002). Seedling infection assay for resistance to *Sclerotium cepivorum* in Allium species. New Zealand Plant Protect. 55:193-196.
- Katan J (1987). Soil Solarization, in Innovative Approaches to Plant Disease Control, Edited by Chet, I. Wiley Interscience, New York. pp. 77-106.
- Kay SJ, Stewart A (1994). Evaluation of fungal antagonists for control of onion white rot in soil box trials. *Plant Pathol.* 43:371-377.
<http://dx.doi.org/10.1111/j.1365-3059.1994.tb02698.x>
- Keusgen M (2002). Health and Alliums, In H.D. Rabinowitch and L. Currah, eds. Allium crop science: Recent advances. CAB International, Wallingford, UK.
- Krest T, Keushen M (1999). Quality of herbal remedies from *Allium sativum*: differences between allinase from garlic powder and fresh garlic. *Planta. Med.* 65:139-143.<http://dx.doi.org/10.1055/s-1999-13975> PMID:10193205
- Krest I, Keusgen M (1999). Quality of herbal remedies from allium sativum: differences between allinase from garlic powder and fresh garlic *Planta Medica* 65(2): 139-143. <http://dx.doi.org/10.1055/s-1999-13975> PMID:10193205
- Leggett ME, Rahe JE (1985). Factors affecting the survival of sclerotia of *Sclerotium cepivorum* in the Fraser Valley of British Columbia. *Ann. Appl. Biol.* 106:255-263.<http://dx.doi.org/10.1111/j.1744-7348.1985.tb03115.x>
- Lo CT, Nelson EB, Hayes CK, Harman GE (1996). Ecological studies of transformed *Trichoderma harzianum* strain 1295-22 in the rhizosphere and on the phylloplane of creeping bentgrass. *Phytopathology*, 88: 129-136.
<http://dx.doi.org/10.1094/PHYTO.1998.88.2.129> PMID:18944981
- Maude RB (2006). Onion Diseases in B. M. Cooke, D. G. J. B. Kaye and Springer, eds. *Epidemiol.* 57:1007.
- Melero-Vara JM, Prados-Ligero AM, Basallote-Ureba MJ (2000). Comparison of physical, chemical and biological methods of controlling garlic white rot. *Eur. J. Plant Pathol.* 106:581-588.<http://dx.doi.org/10.1023/A:1008777706814>
- Metcalfe DD, Wilson CR (2001). The process of antagonism of *Sclerotium cepivorum* in white rot affected onion roots by *Trichoderma koningii*. *Plant Pathol.* 50:249-257.
<http://dx.doi.org/10.1046/j.1365-3059.2001.00549.x>
- Metcalfe DA, Wilson CR (1999). Histology of *Sclerotium cepivorum* infection of onion roots and the spatial relationships of pectinases in the infection process. *Plant Pathol.* 48:445-452.<http://dx.doi.org/10.1046/j.1365-3059.1999.00370.x>
- Mirandaa A, Herrera Estrellab A, Pen˜ a Cabralesa JJ (2006). Colonization of the rhizosphere, rhizoplane and endorhiza of garlic (*Allium sativum* L.) by strains of *Trichoderma harzianum* and their capacity to control allium white-rot under field conditions. *Soil Bio Biochem.* 38: 1823-1830.
<http://dx.doi.org/10.1016/j.soilbio.2005.11.036>
- Mueller S, Hembree K, Molinar R, Schwankl L (2006). Garlic white rot Trial, 2005/2006 progress report. Available online at <http://cagarlicandonion.com/page/1003/resources.html>.
- Nabulsi I, Al-Safadi B, Ali NM, Arabi, MIE (2001). Evaluation of some garlic (*Allium sativum* L.) mutants resistant to white rot disease by RAPD analysis. *Ann. Appl. Biol.* 138:197-202.
<http://dx.doi.org/10.1111/j.1744-7348.2001.tb00102.x>
- New Zealand Agrichemical Manual (2005). Published by AgriMedia Limited, Christchurch. Publications, Oxford, pp. 187-191.
- Porter JI, Merriman PR (1983). Effects of solarization of soil on nematode and fungal pathogens at two sites in Victoria. *Soil Biol. Biochem.* 15(1):39-44. [http://dx.doi.org/10.1016/0038-0717\(83\)90116-5](http://dx.doi.org/10.1016/0038-0717(83)90116-5)
- Prados-Ligero AM, Bascon-Fernandez J, Calvet-pinos C, Corpas-Hervias C, Ruiz AL, Melero-vara JM, MJB (2002). Effect of different soil and clove treatments in the control of white rot of onion. *J. Appl. Biol.* 140:247-253.
- Pung H, Cross S, Florissen P, MacLeod I (2007). Investigating and developing fungicide options for onion white rot control in Australia. Peracto Pty Ltd Publications.
- Rubatzky VE, Yamaguchi M (1997). World vegetable, In Chapman and hall, (eds.) Principles, production and nutritive values, 2nd edition. International thomsonpublishing New York. USA. P. 843.
- Satour MM, Abdel-Rahim MF, El-Yamani T, Radwan A (1989). Soil solarisation in onion fields in Egypt and Israel: short- and long-term effects. *Acta Hortic.* 255:151-159.
- Sovova M, Sova P (2004). Pharmaceutical importance of *Allium sativum* L., Hypolipemic effects *in-vitro* and *in-vivo* (in Czech). *Ceska Slov farm.* 53 (3):117-123.PMID:15218732
- Stewart A, Fullerton RA (1991). Additional studies on the chemical control of onion white rot (*Sclerotium cepivorum* Berk.) in New Zealand. *New Zealand J. Crop Hortic. Sci.* 19:129-134.<http://dx.doi.org/10.1080/01140671.1991.10421790>
- Stewart A, McLean KL (2007). Biological controls of onion white rot. Chapter 6 in Biological control of plant diseases, 2007, The Haworth Press, Inc. pp. 123-148.
- Stewart A, McLean KL (2007). Biological control of plant diseases, Biological control of onion white The Haworth press. rot, 6:123-148.
- Stewart A, Fullerton RA (1991). Additional studies on the control of onion white rot (*Sclerotium cepivorum* Berk) in New Zealand. *J. New Zealand. Crop Hort. Sci.* 19:129-134.<http://dx.doi.org/10.1080/01140671.1991.10421790>
- Tamire Z, Cameda F, Sakuja PK, Seid A (2007). Association of white rot (*Sclerotium cepivorum*) of garlic with environmental factors and cultural practices in the North Shewa highlands of Ethiopia.
- Tyson JL, Fullerton RA, Stewart A (1999). Changes in the efficacy of fungicidal control of onion white rot. *Proceedings of the 52nd New Zealand Plant Protection Conference.* pp. 171-175.
- Tyson JL, Fullerton R, Elliott GS, Reynolds PJ (2000). Use of disulphide for The commercial control of *Sclerotium cepivorum*. *New Zealand J. Plant Protect.* 53:393-397.
- Ulacio-Osorio D, Zavaleta-Mejia E, Martinez-Garza A, Pedroza-Sandoval A (2006). Strategies for management of *Sclerotium cepivorum* Berk. in garlic. *J. Plant Pathol.* 88(3):253-261.
- Utkhede RS, Rahe JE (1980). Biological control of onion white rot. *Soil Biol. Biochem.* 12:101.
- Utkhede RS, Rahe JE, Coley-Smith JR, Van der Meer QP, Brewer JG, Criscola V (1982). Genotype-environment interactions for resistance to onion white rot. *Can. J. Plant Pathol.* 4:269-271.<http://dx.doi.org/10.1080/07060668209501295>
- World Health Organization (2004). The WHO recommended classification of pesticides by hazard and guidelines to classification. ISBN: 92 4 154663 8. ISSN: 1684-1042.
- Zeray S (2011). Distribution and Management of Garlic White Rot in Northern Ethiopia: Distribution, Significance and Management of Garlic White Rot in Northern Ethiopia, Ambo University, Ambo Ethiopia.
- Zeray S, Mohammed Y (2013). Searching and Evaluating of Cost Effective Management Options of Garlic White rot (*Sclerotium cepivorum* Berk) in Tigray, Northern Ethiopia. *J. Plant Pathol. Microbiol.* 4:189.
- Zewide T, Fininsa C, Sakuja PK (2007a). Management of white rot (*Sclerotium cepivorum*) of garlic using fungicides in Ethiopia. *Crop Prot.* 26:856-866.<http://dx.doi.org/10.1016/j.cropro.2006.08.017>
- Zewide T, Fininsa C, Sakuja PK, Ahmed S (2007). Association of white rot (*Sclerotium cepivorum*) of garlic with environmental factors and cultural practices in the North Shewa highlands of Ethiopia. *Crop Protect.* 26:1566-1573. <http://dx.doi.org/10.1016/j.cropro.2007.01.007>

Full Length Research Paper

Plasmid profile of bacterial isolates from fertilizer amended tropical agro soils

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This study assessed the profile of plasmids in the culturable heterotrophic bacterial isolates from organic swine waste (SW) and inorganic (NPK) fertilizer amended tropical soils of the Niger Delta region in Nigeria. Plasmid DNA was extracted and analyzed by agarose gel electrophoresis. Plasmid analysis of 70 fertilizer utilizing bacterial isolates revealed the presence of plasmids with molecular weights ranging from 4.0 to 40.1kb. Twenty two (31%) out of the seventy isolates carried at least one kind of plasmid while forty eight (69%) possessed none. Seventeen (77%) of the isolates with plasmids had one plasmid each of molecular weight approximately 23.1, 25.2 or 35.7kb. Five (23%) possessed two plasmids each of molecular weight approximately 23.1 and 40.1kb, 4.3 and 32.7kb, 4.0 and 40.1kb, 23.5 and 40.1kb or 23.1 and 40.1kb respectively. Bacterial isolates taken from organic fertilizer- amended plots showed highest plasmid incidence (38%) whereas isolates from the control or non- amended plot showed the lowest plasmid incidence (25%). Statistical inferences revealed that plasmid carriage in the bacterial isolates were independent of fertilizer treatments.

Key words: Fertilizer amendments, bacterial isolates, gel electrophoresis, plasmid incidence, molecular weight.

INTRODUCTION

Arable lands or soil considered fit for growing crops (agro soils) contain natural nutrients. However, these nutrients are not readily available to the plants since they are being released in small portions through soil microbial activities (Girvan et al., 2003). Fertilizer amendments are added to

improve or balance the soil nutrients, enhance soil quality and sustainability of the ecosystem. Soil microorganisms act as catalyst in biochemical transformations in soil, playing vital roles in maintaining soil fertility and plant yields. They exert critical effects on ecological stability

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and biological productivity of many fields, forest and grassland ecosystems (Havlin et al., 2009). Recently, research interest are focused on the introduction of sustainable management practices such as cropping systems, fertilizer application, cultivation practices, soil organic amendments and pesticide application in agriculture to maintain soil quality and productivity while minimizing the negative effects on the environment and soil resources (Sayali et al., 2012; Weiss et al., 2012; Ndubuisi-Nnaji et al., 2010). Several studies (Igwo-Ezikpe et al., 2010; Okoro et al., 2009; Coral and Karagoz 2005; Janniere et al., 1993; Okpokwasili et al., 1986) have associated plasmids with the metabolism and degradation of organic compounds like Poly Aromatic Hydrocarbons, Polychloro-biphenyl and pollutants or chemicals like pesticides, ammonium, Nitrous oxide, Phosphates and Carbamates. Although, most genes involved in these degradations have been shown to be plasmid-mediated, strains of *Rhodococcus* and *Pseudomonas* in which all the genes necessary for catabolism are chromosomally-borne have been reported (Takizawa et al., 1994; Bosch et al., 2000).

Plasmids generally carry genes that confer a selective advantage to their host in a specific environment; they increase access to the horizontal gene pool for adaptive traits that may be important in the overall physiology and survival of many bacteria (Obayori and Salam, 2010). Degradative plasmids, can mediate in the utilization of fertilizer amendments as they carry genes that can degrade chemical compounds and fix nitrogen (Okoro et al., 2009; Lipps, 2008). Some ecological studies (Igwo-Ezikpe et al., 2010; Obayori and Salam, 2010; Zhao and Zhou, 2005) have determined the incidence of plasmids in natural populations of terrestrial bacteria but few reports have dealt with the involvement of plasmids in degradation by bacterial populations of an agro ecosystem. This study was therefore aimed at evaluating the plasmid incidence, profiles and carriage frequencies of bacterial isolates from organic swine waste (SW) and inorganic (NPK) fertilizer amended tropical agro soils.

MATERIALS AND METHODS

Experimental site

The experimental plot was established in the University of Uyo Commercial and Research farm in use Offot, Uyo Local Government Area of Akwa Ibom State, South-South Nigeria. The geographical position of the study site is at 5.0167°N latitude and 7.9667°E longitude and situated at an altitude of 38.1m above Sea level. The climate is tropical and the rainy season starts from April and continue till late October while the dry season is from November to March. The soil of the experimental site was sandy loam (72.60%) with moderate permeability, silt (9.04%) and clay (18.36%). The soil pH values ranged from 4.3 to 4.5.

Experimental design

Field and laboratory studies were carried out to evaluate the

plasmid profile of bacterial isolates from fertilizer amended tropical agro soils. The experimental layout was a randomized complete block design (RCBD) of Anderson and Ingram (1993) with slight modifications. It comprised of four experimental blocks or replicates with four treatments namely: Control (None), Organic fertilizer (SW), Inorganic fertilizer (NPK) and combined organic and inorganic fertilizer (SW+NPK). The experimental blocks were located in areas with good drainage and topography and measured 15 × 15 m each. The dimensions of the individual plots within each block were 7 × 7 m with 1 to 2m line spacing between rows.

Sample sources and properties

The commercial grade of NPK 15:15:15 (inorganic fertilizer) used for the study was obtained from the Fertilizer Procurement Unit of the Akwa Ibom State Ministry of Agriculture, Uyo while the Swine waste SW (organic fertilizer) was collected from the Piggery Unit, Crop Science and Animal Production Department, University of Uyo Commercial and Research farm, Use Offot, Uyo, Akwa Ibom State, Nigeria. The swine waste was air dried before use. The rate of application per plot was calculated based on the recommended rates of field application per hectare (Havlin et al., 2009; Edem, 2007).

Field sampling and processing

Following the amendment of the soils with the different fertilizer treatment, soil samples were collected aseptically using a soil auger (sterilized by washing and swabbing with an alcohol-soaked cotton wool) at monthly intervals for a period of one year. On each sampling date, about 4 to 5 representative soil samples were taken randomly from the surface soil (0 to 30 cm depth) of each treatment plot, mixed and bulked into a composite sample. The soil samples were placed in polyethylene bags and labeled according to the fertilizer treatment for each block as follows: NONE, NPK, SW and SW + NPK respectively. The labeled samples were taken to the laboratory in ice cold packs for microbiological assays. Where the samples were not analyzed same day, they were stored at 4-5°C in a refrigerator and used subsequently.

Isolation and enumeration of culturable heterotrophic bacteria

The total heterotrophic soil bacteria was enumerated after surface-plating on nutrient agar, supplemented with Nystatin to inhibit fungal growth according to the methods of Parham et al. (2003). Discrete colonies were further purified by sub-culturing on Nutrient agar and Mac Conkey agar respectively and incubated at 37°C for 18 to 24 h. They were identified using morphological and biochemical studies such as Gram's reaction, slide agglutination, coagulase, catalase, oxidase, citrate utilization and sugar fermentation tests were performed. Further identification of the isolates was done according to the methods of Holt et al. (1994), Barrow and Feltham (2003) and Etok et al. (2004). To screen for their ability to utilize the fertilizer amendments, isolates were cultured on sterilized solid mineral salt agar (MSM) plates containing swine waste and NPK fertilizers (0.01%^{w/w}) respectively as sole carbon and energy source (Lalfakzuala et al., 2008). Culture plates and control plates (devoid of fertilizers) were incubated at room temperature for 5 to 7days and observed for growth.

Plasmid DNA isolation and profiling

Plasmid studies was carried out at molecular biology and

biotechnology laboratory, Nigerian Institute for Medical Research (NIMR) Yaba, Lagos. Seventy bacterial isolates that showed pronounced growth and good colonial morphology on mineral salt-fertilizer media were further screened for the presence or absence of plasmids to ascertain the involvement of plasmids in the utilization of fertilizer by the isolates. The plasmid DNA extraction was by the TENS Mini Prep method modified by Zhou et al. (1990).

Plasmid profiling was done by agarose gel electrophoresis. A DNA molecular weight marker (Hind III digest of λ - DNA and/or Ori kb - Promega, USA) was used as a standard. Gel electrophoresis was carried out in a horizontal tank at a constant voltage of 60V (Thermo EC machine, CBS scientific, USA) for 90 min. Plasmid DNA bands were viewed by fluorescence of bound ethidium bromide under a short wave ultraviolet (UV) light with a photo documentation system (Clinix, Japan) and photographed. The DNA bands were matched with those for λ DNA Hind III digest and/or Ori kb molecular weight maker. The approximate molecular weight of each plasmid was obtained by extrapolation on graphical plots of molecular weight of marker against the distance traveled by the respective band.

Statistical analysis

Statistical inferences were used to assess the plasmid carriage frequencies in the isolates and the relationship between plasmid carriage in the bacterial isolates and the fertilizer treatments. Study data was analyzed using SPSS statistical analysis package version 17.0. ANOVA was performed for bacterial population data, treatment options and their interactions. Chi square (χ^2) test of independence was performed to ascertain the relationship between plasmid carriage in bacterial isolates and the different fertilizer treatments. Result were considered significant at $P < 0.05$ (Ubom, 2004)

RESULTS AND DISCUSSION

The results from this study revealed the prevalence of culturable heterotrophic bacterial isolates in the fertilizer-amended soils and the control. Diverse species of microorganisms namely; *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Pseudomonas*, *Staphylococcus*, *Micrococcus* and *Corynebacterium* were isolated from both the fertilizer-amended and non-amended tropical agro soils. The abundance of a variety of microorganisms in these soils which are continuously cultivated thus suggests the adaptive abilities of these organisms to the environmental stress and agricultural inputs. Such mechanism of genetic adaptation by microorganisms usually acquired by evolution of metabolic/degradative ability is facilitated by the presence of plasmids (Top and Springeal, 2003). Plasmids are important in the overall physiology and survival of many bacteria as they carry genes that confer a selective advantage to their host in a specific environment. However, their genetic information is not essential to the host as cells that lack them usually function normally (Obayori and Salam, 2010).

Plasmid analysis of the seventy fertilizer utilizing bacterial isolates, revealed the presence of plasmids of different sizes (Table 1) with molecular weights ranging from 4.0 to 40.1 kb. Only twenty two (31%) of the isolates

carried at least one kind of plasmid and forty eight (69%) of the isolates possessed no plasmids. Plasmid profiles revealed that isolates in lanes 1 to 11 (Figure 1) carried one plasmid each of size approximately 23.1kb except for isolates in lanes 8, 9 and 11 whose plasmid size was 25.2kb. Isolates in lanes 14, 15 and 16 (Figure 2) also carried one plasmid each of size approximately 35.7kb. Five of the isolates in lanes 12, 13, 17, 19 and 20 (Figure 2) carried two plasmids each with larger sizes/molecular weight. The largest plasmid size of 40.1kb was carried by *Bacillus megaterium* and *Bacillus subtilis*. These observations were similar to the reports of Kunnimalaiyan and Vary (2005) that many strains of *B. megaterium* carried 4 to 10 plasmids of sizes ranging from 5.4kb to over 165kb. Ohtani et al. (2008) also reported the presence of a 65kb conjugal plasmid pLS20 in *B. subtilis*.

Manure and other organic amendments have been shown to have numerous gross effects on soil microbiology. This study revealed that, the frequency of plasmid-carriage in bacterial isolates was higher in the organic fertilizer-amended soils (SW) (Table 3) than in the control/non-amended (NONE), inorganic (NPK) or combined (SW+NPK) fertilizer-amended treatment plots. Although Chi square (χ^2) test of independence indicated that there exist no relationship between the plasmid carriage in the isolates and the type of fertilizer treatments applied to the soil, Parham et al. (2003) reported that both dairy and swine manures generally increased bacterial population and activity. It was also observed that bacterial isolates taken from the swine waste-amended plots (Table 2), showed the highest plasmid incidence (38%) while isolates from the control or non- amended plots showed the lowest plasmid incidence (25%). The positive effect of organic fertilization accounted for these observations.

Although plasmid carriage in the bacterial isolates was independent of fertilizer treatments applied to soil, the ubiquity of bacterial plasmids in the heterotrophic population of tropical agro soils is indicative of the role of plasmids in the adaptation of these bacterial isolates to life in continuously cultivated and fertilizer amended soils. Identification of the plasmid-carrying isolates revealed approximately 75% of them to be Gram positive rods, particularly of the *Bacillus* genera. This corroborates earlier observations (Kunnimalaiyan et al., 2001; Igwilo-Ezikpe et al., 2010) that several species of Gram positive bacteria carry multiple plasmids which serve as adaptive mechanism, especially those belonging to the genera *Staphylococcus*, *Streptococcus*, *Lactococcus*, *Bacillus* and *Corynebacterium*.

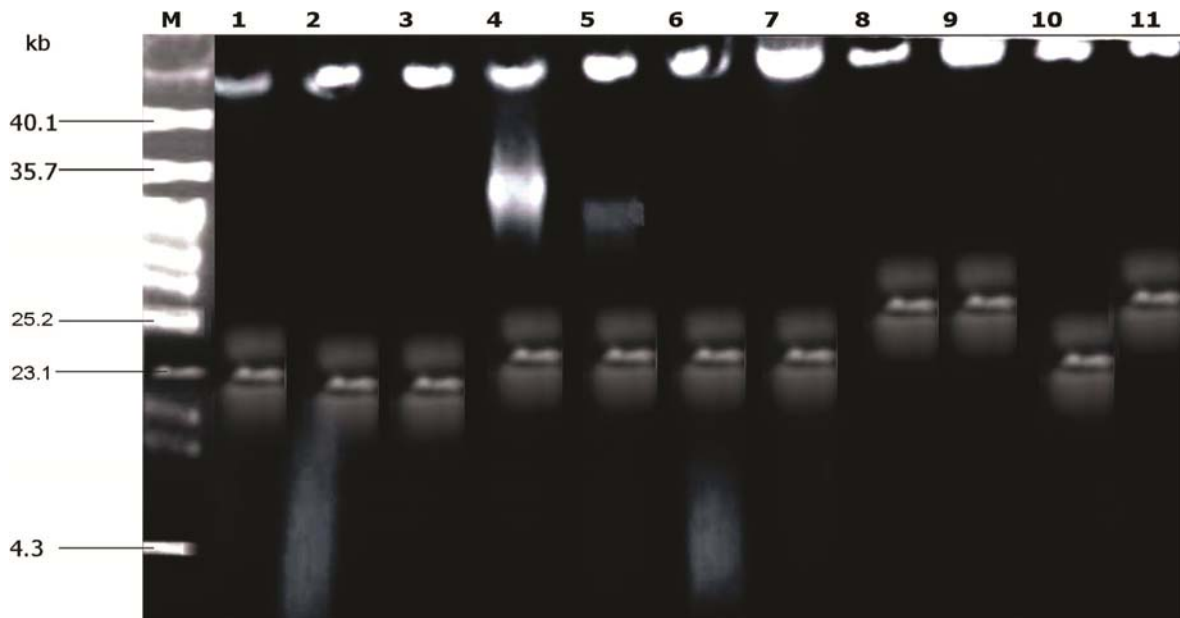
Conclusion

Plasmid incidence in about 31% of these bacterial isolates illustrates their adaptability to environmental stress induced by possible overuse or misuse of soil amendments.

Table 1. Molecular weight of plasmid DNA from screened bacterial isolates.

Isolate Code	Host organism	Number of Plasmids	Molecular weight of plasmids (kb)
ISN 1	<i>Bacillus megaterium</i>	1	23.1
ISN 5	<i>Acinetobacter calcoaceticus</i>	1	23.1
ISN 8	<i>Bacillus megaterium</i>	1	23.1
ISN 18	<i>Bacillus subtilis</i>	1	23.1
ISN 19	<i>Bacillus subtilis</i>	1	23.1
ISN 24	<i>Staphylococcus aureus</i>	1	23.1
ISN 30	<i>Bacillus cereus</i>	1	23.1
ISN 33	<i>Bacillus subtilis</i>	1	25.2
ISN 44	<i>Rhodococcus sp.</i>	1	25.2
ISN 45	<i>Bacillus cereus</i>	1	23.1
ISN 52	<i>Bacillus subtilis</i>	1	25.2
ISN 53	<i>Bacillus megaterium</i>	2	40.1, 23.1
ISN 54	<i>Bacillus megaterium</i>	2	40.1, 23.1
ISN 56	<i>Pseudomonas sp</i>	1	35.7
ISN 57	<i>Rhodococcus sp</i>	1	35.7
ISN 58	<i>Micrococcus lylae</i>	1	35.7
ISN 61	<i>Bacillus subtilis</i>	2	40.1, 23.5
ISN 62	<i>Bacillus megaterium</i>	1	40.1
ISN 63	<i>Staphylococcus scheiferi</i>	2	32.7, 4.3
ISN 64	<i>Bacillus megaterium</i>	2	40.1, 4.0
ISN 68	<i>Staphylococcus intermedius</i>	1	25.2
ISN 69	<i>Corynebacterium ammoniagenes</i>	1	23.1

ISN = Isolate Code

**Figure 1.** Agarose gel electrophoresis of plasmid DNA from fertilizer utilizing bacteria isolates. Lane 1= *Bacillus megaterium*, 2= *Acinetobacter calcoaceticus*, 3= *Bacillus megaterium*, 4= *Bacillus subtilis*, 5= *Bacillus subtilis*, 6= *Staphylococcus aureus*, 7= *Bacillus cereus*, 8= *Bacillus subtilis*, 9 = *Rhodococcus sp.* 10= *Bacillus cereus*, 11= *Bacillus subtilis*.

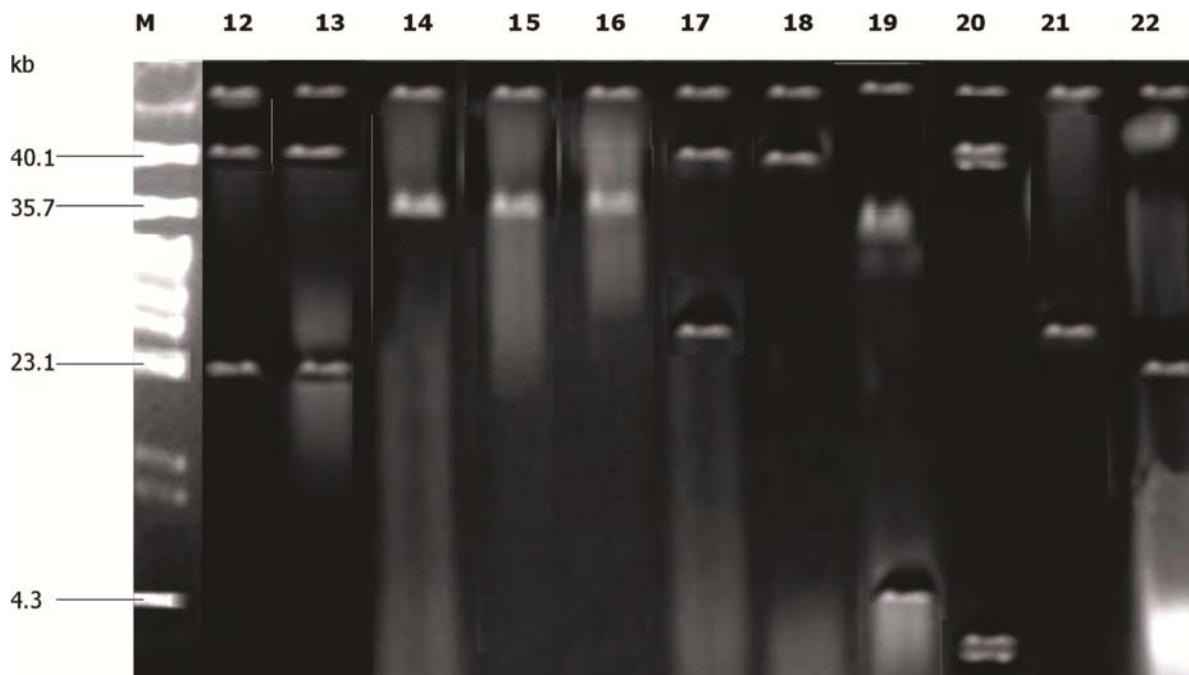


Figure 2. Agarose gel electrophoregram of plasmid DNA from fertilizer utilizing bacteria isolates. Lane 12= *Bacillus megaterium*, 13= *Bacillus megaterium*, 14= *Pseudomonas sp.* 15= *Rhodococcus sp.*, 16= *Micrococcus lylae*, 17= *Bacillus subtilis*, 18= *Bacillus megaterium*, 19= *Staphylococcus scheiferi*, 20= *Bacillus megaterium*, 21= *Staphylococcus intermedius*, 22= *Corynebacterium ammoniagenes*.

Table 2. Percentage of plasmid carrying bacterial isolates from each fertilizer-amended soil and control.

Fertilizer Treatments	No. of isolates Screened	No. of isolates with plasmids	% of isolates with plasmids
NONE	12	3	25
FYM	21	8	38
NPK	19	6	31
NPK+FYM	18	5	28

NONE = No fertilizer amendment (control); SW = Farmyard Manure/Organic Fertilizer amendment; NPK = Inorganic Fertilizer amendment SW + NPK = Combined Organic and Inorganic Fertilizer amendment

Table 3. Frequency of plasmid carriage in bacteria isolated from different treatments.

Fertilizer Treatments	No. of Isolates carrying Plasmids	No. of Isolates not carrying Plasmids	Total No. from each Treatment
NONE	3 (3.77)	9 (8.23)	12
FYM	8 (6.6)	13 (14.4)	21
NPK	6 (5.97)	13 (13.03)	19
NPK+FYM	5 (5.66)	13 (12.34)	18

NONE = No fertilizer amendment (control); SW = Organic Fertilizer amendment; NPK = Inorganic Fertilizer amendment; SW +NPK = Combined Organic and Inorganic Fertilizer amendment.

It also underscores the critical effects these microorganisms could have on the ecological stability and

biological productivity of the agro ecosystem, as well as their potentials for use in bioremediation and eco restoration

in order to ensure environmental sustainability and national development.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

- Anderson JM, Ingram JSI (1993). Tropical soil biology and fertility: A Handbook of Methods. CAB International, Oxford.
- Barrow IG, Feltham KAR (2003). Cowan and Steel's Manual for the identification of Medical Bacteria. Cambridge University Press: Edinburgh.
- Bosch R, Garcia-Valdes E, Moore ERB (2000). Complete nucleotide sequence and evolutionary significance of a chromosomally encoded naphthalene degradation lower pathway from *Pseudomonas stutzeri* ANIO. *Gene* 245:65-74. [http://dx.doi.org/10.1016/S0378-1119\(00\)00038-X](http://dx.doi.org/10.1016/S0378-1119(00)00038-X)
- Coral G, Karagoz S (2005). Isolation and characterization of phenanthrene degrading bacteria from a petroleum refinery soil. *Ann. Microbiol.* 55(4):255-259.
- Edem SO (2007). Soil: The Dynamic System. Minder International Publishers, Uyo, Akwa Ibom State.
- Etok CA, Udo SM, Eja M (2004). General microbiology practical manual. Abison Printing Press. Calabar, Nigeria.
- Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS (2003). Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Appl. Env. Microbiol.* 69(3):1800-1809.<http://dx.doi.org/10.1128/AEM.69.3.1800-1809.2003> PMID:12620873 PMCID:PMC150080
- Havlin JL, Beaton JD, Tisdale SL, Nelson WL (2009). Soil Fertility and Fertilizers- An Introduction to Nutrient Management, 7th ed. Pearson Education Inc. New Jersey, USA.
- Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilkins: Baltimore; pp. 532-551.
- Igwo-Ezikpe MN, Okpuzor J, Awodele O, Nwaokorie FO, Fowora MA, Akinbo MO (2010). Prevalence of polycyclic aromatic hydrocarbons (PAHs) degrading bacteria in contaminated tropical soil in Lagos, Nigeria: Involvement of plasmid in degradation. *Intl. J. Bio. Chem. Sci.* 4(6):2133-2145.
- Janniery L, Gruss A, Ehrlich S (1993). Plasmids. In A. Sonenshein, J. Hoch and R. Losick (ed). *Bacillus subtilis* and other Gram Positive Bacteria. America Society for Microbiology, Washington, D.C. pp. 625-645.
- Kunnimalaiyan M, Vary PS (2005). Molecular characterization of plasmid PBM 300 from *Bacillus megaterium* QM B1551 *Appl. Environ. Microbiol.* 71(6):3068-3076.<http://dx.doi.org/10.1128/AEM.71.6.3068-3076.2005> PMID:15933003 PMCID:PMC1151835
- Kunnimalaiyan M, Steveson DM, Zhou Y, Vary PS (2001). Analysis of the replicon region and identification of an RNA operon on PBM 400 of *Bacillus megaterium* QM B1551. *Mol. Microbiol.* 39:1010-1021.<http://dx.doi.org/10.1046/j.1365-2958.2001.02292.x>
- Lalfakzuala R, Kayang H, Dkhar MS (2008). The effects of fertilizer on soil microbial components and chemical properties under leguminous cultivation. *Am-Eurasian J. Agric. Environ. Sci.* 3(3):314-324.
- Lipps G (ed) (2008). Plasmids:Current Research and Future Trends. Caister Academic Press pp 264.18/12/2010 (internet) <http://www.biotecharticles.com>.
- Ndubuisi-Nnaji UU, Adegoke AA, Ogbu HI, Ezenobi NO, Okoh AI (2010). Effect of long-term organic fertilizer application on soil microbial dynamics. *Afr. J. Biotechnol.* 10(4):556-559.
- Obayori OS, Salam LB (2010). Degradation of polycyclic aromatic hydrocarbons: Role of plasmids. *Sci. Res. Essays* 5(25):4093-4106.
- Ohtani N, Sata M, Tomita M, Itaya M (2008). Restriction on conjugational transfer of pLS20 in *Bacillus subtilis* 168 *Biosci. Biotechnol. Biochem.* 72(a):2472-2475.
- Okoro CC, Amund OO, Eliora ER (2009). Plasmids of phenanthrene and dibenzothiophene degrading bacteria isolated from produced water samples of oil production operations. *Intl J. Chem. Biol. Sci.* 3(2):186-191.
- Okpokwasili GC, Somerville SC, Grimes DJ, Colwel RR (1986). Plasmid-associated phenanthrene degradation by Chesapeake Baysediment bacteria. *Deuxime Colloque International de Bacteriologie Marine, Actes de Colloques* 3:601-610.
- Parham JA, Deng SP, Da HN, Sun HY, Raun WR (2003). Long-term cattle manure application in soil II. Effect on soil microbial populations and community structure. *J. Biol. Fert. Soils* 38:209-215.<http://dx.doi.org/10.1007/s00374-003-0657-7>
- Sayali RN, Annika AD, Meeta B, Jossy V, Naresh C (2012). Isolation, characterization and identification of pesticide tolerating bacteria from garden soil. *Eur. J. Exptl. Biol.* 2(5):1943-1951.
- Takizawa N, Kaida N, Torigoe S, Mortitani T, Sawada T, Satoh S, Kiyohara S (1994). Identification and characterization of genes encoding polycyclic aromatic hydrocarbon dioxygenase and polycyclic aromatic hydrocarbon dihydrodiol dehydrogenase in *Pseudomonas putida* OUS82. *J. Bacteriol.* 176:2444-2449. PMID:8157615 PMCID:PMC205371
- Top EM, Springeal D (2003). The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds. *Curr. Opin. Biotechnol.* 14:262-269. [http://dx.doi.org/10.1016/S0958-1669\(03\)00066-1](http://dx.doi.org/10.1016/S0958-1669(03)00066-1)
- Ubom RM (2004). Biometry: a functional approach. 1st ed. Abaam Publishing Company, Uyo, Nigeria.
- Weiss M, Haufe J, Carus M, Brandão M, Bringezu S, Hermann B, Patel MK (2012). A review of the environmental impacts of biobased materials. *J. Ind. Ecol.* 16:S169-S181.<http://dx.doi.org/10.1111/j.1530-9290.2012.00468.x>
- Zhao Y, Zhou LW (2005). DNA extraction from soil for molecular microbial community analysis. *J. Agro-environ. Sci.* 24:854-860.



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