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Preface

We would like to present, with great pleasure, the inaugural volume-7, Issue-11, November 2021, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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Tree Bean (*Parkia Roxburghii*) declined in Mizoram

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Abstract— *Tree bean (Parkia roxburghii) is an important leguminous cash crop in Mizoram and other North Eastern Region of India. However, its production is threatened by several abiotic and biotic factors, especially insects and diseases. Declined of tree bean was frequently observed and reported in all the districts of Mizoram since 90's which caused serious setback for tree bean growers. However, there were no systematic studies or research conducted to assess the role of insect pests on declining tree bean in Mizoram. The present investigation was carried out in Thiltlang, Darzo, Tuipui D, Rotlang East and Hnahthial of the then Lunglei district, Sangau of Lawngtlai district and Siaha of Siaha district and on road side plantation of NH-54 from Aizawl to Tipa and World Bank road from Aizawl to Lunglei, Mizoram, India during October 2017 to October 2020. The present works was aimed at studying the insect species associated with this crop in the states and also constitute an important baseline data for the design and implementation of IPM strategies for Tree bean protection in Mizoram. The frequently associated insect with partial or complete declined of tree bean was identified as Ambrosia and bark beetles, Blepephaeus succinctor and Xystrocera globosa. In addition, Ambrosia beetles (Curculionidae, Scolytinae) and Blepephaeus succinctor (Coleoptera: Cerambycidae: Lamiinae) was reported for the first time attacking and causing Tree bean decline in Mizoram.*

Keywords— Ambrosia and bark beetles, Blepephaeus succinctor, Xystrocera globosa, Partial or complete decline, Integrated Pest Management, Mizoram.

I. INTRODUCTION

Tree bean (*Parkia roxburghii*) is economically important multipurpose fast growing deciduous leguminous tree vegetable of Mizoram and other north eastern states of India. It is distributed in India, Bangladesh, Myanmar, Java, Thailand, Egypt and the Malaysian region. In North Eastern India, tree bean is found growing wild throughout the region. It is known as Zawngtah in Mizo language and the trees are mostly grown in jhumland, roadside, orchard, and backyard garden. Almost every part of the plants an i.e flower, tender leaves, tender pods and mature seeds are eaten as vegetables and provide a good source of nutrients. The mature seeds are useful against food allergy, diarrhea and dysentery. Traditionally, the bark and fruits are prescribed to check excessive bleeding during menstruation and the juice of the green rind of the pod is applied to fresh cuts, scabies and itching. In addition, the seeds are eaten by various wild animals and birds. The matured pod is one of the delicacies of the tribal people of the region and fetching high market price. Tree bean decline have been frequently observed in Mizoram since 90's but since the sudden death have been confined in few locations, no proper studies on the decline have been carried out. Thangjam 2006 reported that the problems of die-back symptoms in *P. timoriana* have also been found to be associated with the infestation of *Anoplophora glabripennis* (Motchulsky) commonly known as Asian longhorned beetle and Recently, sudden decline of tree bean was reported from various parts of Manipur, Nagaland, Mizoram and Meghalaya, which seriously affected the socio-economic situation of the growers (Thangjam and Sahoo 2012). Preliminary investigation on the reasons behind the decline of tree bean was carried out during 2012-2013 in all the Krishi Vigyan Kendra of Mizoram under the direction of Director of Agriculture (Research and Education), Government of Mizoram but no proper conclusion was made. Recently, it was found that the sudden death or decline of tree bean in Mizoram was frequently associated with pathogen identified as *Botrydiplodia theobromae* and also some small unidentified bark borers infesting the tree (Ratankumar Singh et al 2018). In Manipur, tree bean decline was linked to insect stem borer, *Bactocera* sp. (Surendranath, 2007). Rajesh et al 2012 reported *Verticilium dahlae* causing seedling wilt of tree bean. Thangjam et al 2003 reported that *C. cautella* infested tree bean plant in both field and on storage conditions and the larvae was found to feed voraciously on the green kernels. All the stages in the life cycle of the insect were observed on *P. timoriana*. Sinha et al 2017 reported *Lasiodiplodia theobromae* causing dieback in tree bean decline in Northeast India.

Roy et al 2016 reported a large number of insect pests like; Asian long horned beetle (*Anoplophora glabripennis*); bark eating caterpillar (*Indarbela spp*); jassid (*Empoasca kerri*); aphid (*Aphis craccivora*); thrips (*Scirtothrips dorsalis*); green stink bug (*Nezara virudula*); Coreid bug/tur pod bug (*Clavigralla gibbosa*); spotted pod borer (*Cadra cautella*) to be associated with the tree bean. Out of these, Asian long horned beetle, bark eating caterpillar and spotted pod borer are most commonly found associated with declining trees. Moreover, *Verticillium* wilt and collar rot have also been observed in declining tree bean plantation. There were reports on large scale spread of *Verticillium* wilt from Chandel district of Manipur. Collar rot (*Phytophthora* sp.) has been found in valley areas, especially in Imphal West and Bishnupur district of Manipur. (Roy et al., 2016). Diseases and pests *Parkia* species have a number of pests in common with other leguminous trees and shrubs. The stem and bark borers *Xystrocera festiva* and *Cossus subfuscus* can cause severe damage in *Parkia speciosa*, especially at lower elevations in Java. Other pests are the pod borers *Cryptophlebia ombrodelta* and *Mussidia pectinicornella*, and the caterpillars of the leaf feeders *Polyura hebe*, *Eurema blanda* and *Eurema hecabe*. The seeds are relished by a great number of arboreal mammals (Yusuf et al., 2001)

II. MATERIALS AND METHODS:

Tree bean is found associated to numerous insect pest right from nursery stage to full grown stage. However, research on insect pest of this multipurpose tree is very scanty. Thus, the present study examined the insect pest associated to tree bean and its involvement in the sudden decline of this particular tree in Mizoram.

2.1 Study Area

The present investigation was carried out in Thiltlang, Darzo, Tuipui D, Rotlang East and Hnahthial of the then Lunglei district, Sangau of Lawngtlai district and Siahatlah-III of Siaha district, Mizoram, India during October 2017 to October 2020 covering 430 numbers of trees of different ages. In addition, incidences of different insects on tree bean were surveyed and observed randomly on road side plantation of NH-54 from Aizawl to Tipa and World Bank road from Aizawl to Lunglei covering 180 numbers of trees of different ages ranging from 3 to 15 years.

Pest incidence/percentage of infestation: The total no of healthy trees, partially dead and completely dead trees were recorded from the randomly selected trees to work out the percentage of death tree/declined tree. Besides, the basal portions of the selected tree were inspected carefully up to 5ft. to identify borer infestation and holes of different size were counted and recorded. The activity and role of these insect were also studied in field condition.

2.2 Collection and preservation of insects

The emerging adult insects were collected in situ with the help of fine cloth bags covering the cutted logs and also by splitting open the cutted log with axe. The photographs of all the associated insects were taken with a Digital Camera.

The adults were conserved in 70% ethanol or dry (card mounted and pinned) and the immature stages were also preserved in 70% ethanol. The insects collected were sent to the Zoological Survey of India, Kolkata for Identification.

III. RESULTS & DISCUSSION

3.1 Pest incidence/percentage of infestation

The results of the survey conducted in the villages of Thiltlang, Darzo, Tuipui D, Rotlang East Hnahthial, Sangau and Siaha and road side plantation of NH-54 from Aizawl to Tipa and World Bank road from Aizawl to Lunglei reveals that 29.73% (183 numbers) and 38.57% (233 numbers) were completely and partially death respectively. Among the selected villages/town area, Rotlang east village has the highest percentage of completely declined tree (40%) followed by Sangau (34.44%) and Thiltlang (34.16%) whereas Tuipui D village has the lowest percentage of completely declined tree (20%). Besides, Thiltlang village has the highest percentage of semi-declined tree (42.35%) followed by Hnahthial (42%) and Sangau (41.11%) which are at par to each other. In addition, the percentage of semi/partially declined tree was lowest in roadside plantation of NH-54 from Aizawl to Tipa (29%). The overall percentages of completely declined and partially declined tree were 29.73% and 38.57% respectively (Table 1).

TABLE 1
SURVEY AREA AND GENERAL CONDITIONS OF THE TREE

Sl.No	Villages/town/Area	Total Nos. of tree survey	Total Nos. of Completely death tree (Complete declined)	Total Nos. of Partially death tree (Semi-declined)	Total Nos. of Healthy tree	% of Completely declined tree	% of Semi-declined tree
1	Thiltlang	85	29	36	20	34.16	42.35
2	Darzo	75	17	27	31	22.67	36.00
3	Tuipui D	30	6	11	13	20.00	36.67
4	Rotlang East	35	14	14	7	40.00	40.00
5	Hnahthial	50	15	21	14	30.00	42.00
6	Sangau	90	31	37	22	34.44	41.11
7	Siaha	65	20	26	19	30.77	40.00
8	NH-54 (Aizawl to Tipa)	100	33	29	38	33.00	29.00
9	World Bank road (Aizawl to Lunglei)	80	18	32	30	22.50	40.00
	Total	**610**	**183**	**233**	**194**	**29.73**	**38.57**

TABLE 2
AMBROSIA BEETLE AND OTHER BORER INFESTATION IN DIFFERENT LOCATION OF MIZORAM

Sl.No	Villages/town/Area	Total Nos. of tree inspected	Average Nos. of Ambrosia beetle and other bark beetle holes (small/pin hole size) per tree	Average Nos. of other beetles holes (Larger holes) per tree
1	Thiltlang	10	131.25*	5.50
2	Darzo	10	61.70	3.25
3	Tuipui D	10	99.10	-
4	Rotlang East	10	128.25	3.50
5	Hnahthial	10	119.75	4.15
6	Sangau	10	124.70	1.75
7	Siaha	10	98.80	2.00
8	NH-54(Aizawl to Tipa)	10	78.50	-
9	World Bank road (Aizawl to Lunglei)	10	81.80	1.00
	Total	**90**	**102.65**	**2.35**
The holes were counted from ground level up to 5 ft.				

Ambrosia beetle and other borer infestation were observed by counting the numbers of holes present from the ground level up to 5 ft. 10 numbers of trees were randomly selected from 9 locations thereby 90 numbers of trees were covered under the processed. The averaged numbers of Ambrosia beetle and other bark beetle holes was highest in Thiltlang (131.25) followed by Rotlang East (128.25) and Sangau (124.70). The averaged numbers of other beetle (larger holes) was also highest in Thiltlang (5.50) followed by Hnahthial (4.15) and Rotlang East (3.50). The overall average numbers of Ambrosia beetle and other bark beetle holes was 102.65 whereas that of other beetle (larger holes) was only 2.35 (Table 2.).

3.2 Collection and preservation of insects

During the study period 18 species of beetles ranging from few millimeters (Ambrosia beetle and other bark beetles) to few centimeter (long horned beetles), mostly unidentified (Fig. 3) were collected from different locations. Besides, large numbers of different lepidopteran larva (immature stages) were also collected for identifications. Among the insects collected, *Blepephaeus succinctor* was reported for the first time from tree bean and found to be associated to tree bean declined (Fig. 1). This particular insect was mostly found to attacked tree bean age ranging from 2-10 years and caused extensive damaged especially in Thiltlang and Hnahthial areas. *Xystrocera globosa* was also active in Thiltlang, Darzo, Rotlang East and Hnahthial areas (Fig. 2a). Some unidentified borer larvae were also found causing extensive damage to tree bean especially on older trees (Fig. 2b, 2c & 2d).

FIGURE 1: a) Entry point of *Blepephaeus succinctor*. b) larval stage of *B. Succinctor* and c) adult of *B. succinctor* (Coleoptera: Cerambycidae: Lamiinae)

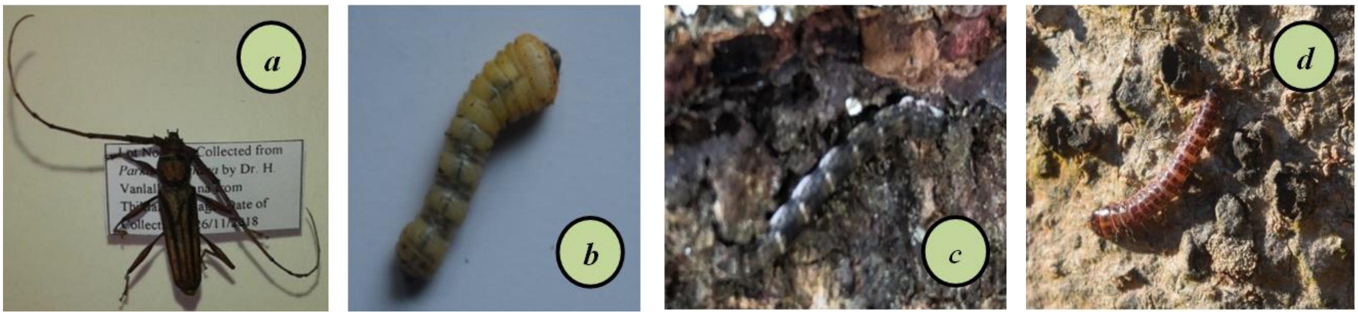

FIGURE 2: a) Adult Xystrocera globosa (Coleoptera: Cerambycidae), b, c & d) Larva of Unidentified borer

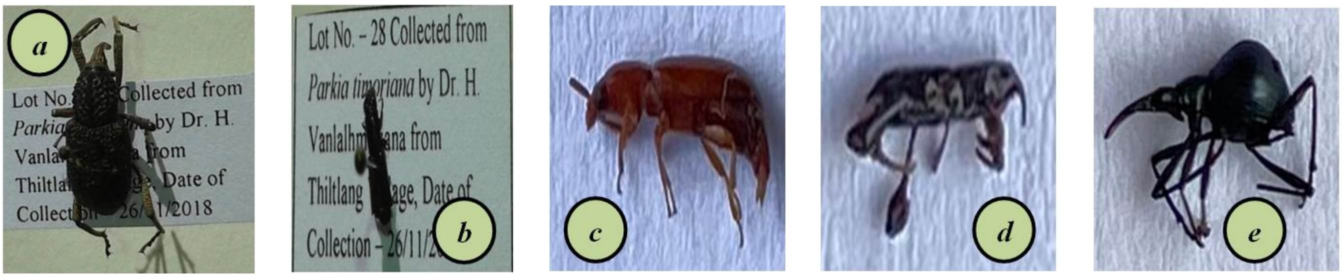

FIGURE 3: Some of the unidentified borer collected from the study sites (a, b, c, d & e)

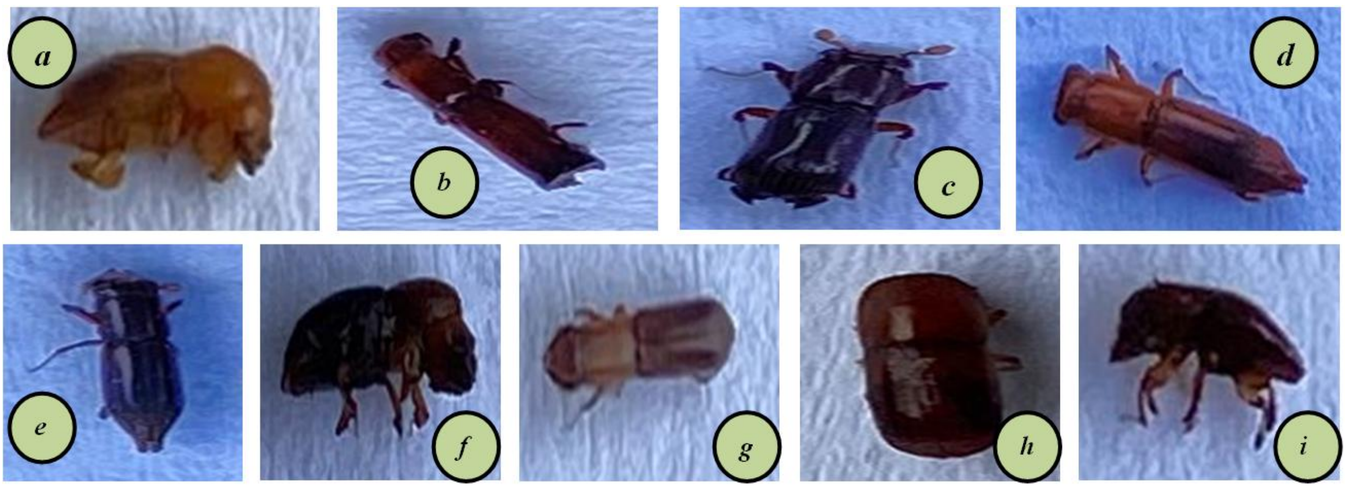

FIGURE 4: Unidentified ambrosia and bark beetle collected from the study sites (a-i)

3.3 Main reasons for declined of tree bean in Mizoram

During the study period, large numbers of insect's species were encountered but identification of the insects was the main problem. Most of the insects species collected was few in numbers and does not play significant role in the declining of tree bean in the region. However, Ambrosia beetles and other bark beetles pose a serious threat and play significant roles in the declining of tree bean in Mizoram. Moreover, *B. succinctor* and *X. globosa* was also found to be very destructive especially to young trees. In some cases, partially declined tree was found regenerated (fig. 5g) after spraying of systemic insecticides and cutting the wilted top portion of the trees. In general, tree bean plants are not well managed like other fruit crops in Mizoram. The seedlings were directly planted without digging pit or maintaining proper spacing and two to three weeding was done per year only without watering nor fertilizer applications. The plant was mostly left unattended till the plant shows sign of declined and when the farmers realized that the trees need due care it was always too late. The infested trees start shedding yellowing leaves and mostly drying up from the top downwards with prominent die back symptoms. Majority of the plant will completely dries up and dies within a few months, but in some cases it takes more than a year. The plant that dies slowly after having wilting symptoms was mostly attacked by insects only but the plants that die faster when cut opens usually shows sign of diseases infections also (Fig 5c). It can be concluded that the vast numbers of Ambrosia and bark beetles act as vector and transmitted pathogens among tree bean which accelerates the dying process of tree bean.

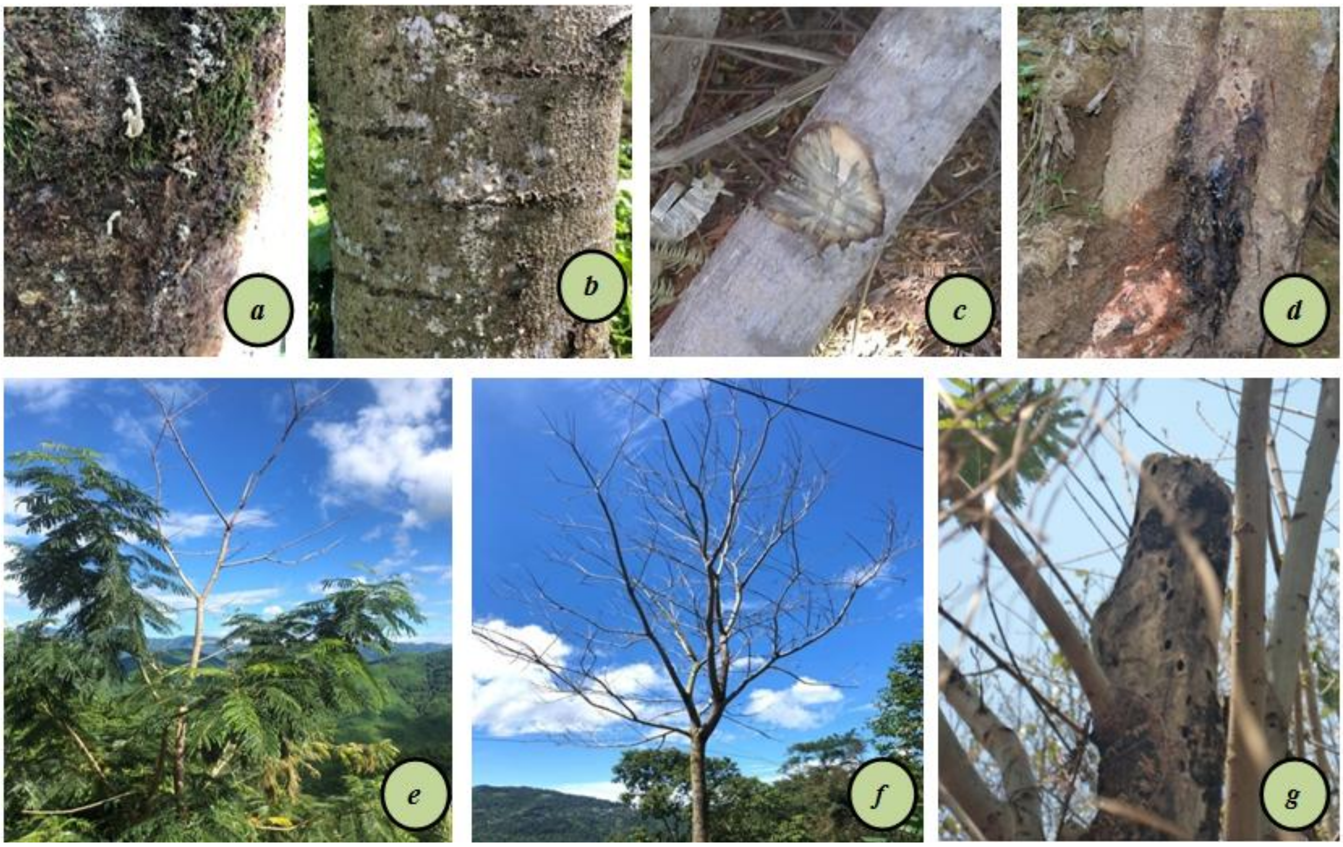

FIGURE 5: Nature of damaged of ambrosia and bark beetles:- a &b) production of trash like saw-dust on the entry point; c) wood staining by fungus; d) gum exudation; e) partial declined; f) complete declined; g) regeneration from partially declined tree

FIGURE 6: Collection of insects; a) In situ collection; b&c) ex-situ collection by bagging the cutted logs.

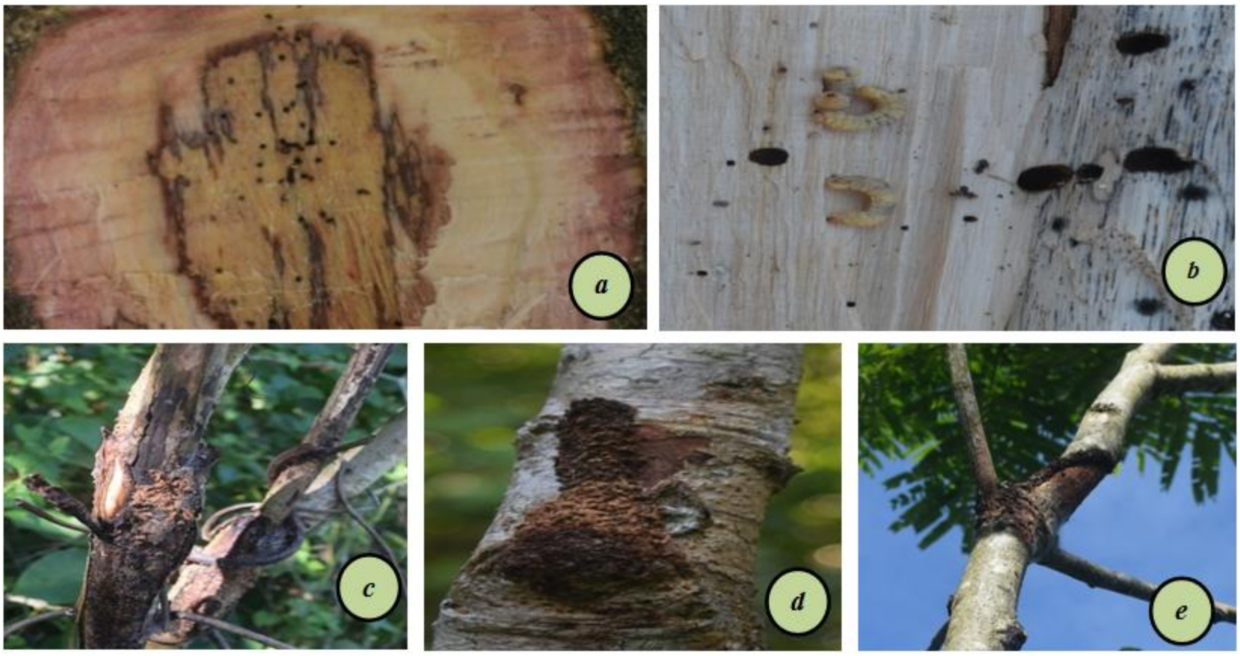

FIGURE 7: Bored holes and sign of different insects; a) bore holes of Ambrosia beetles; b) bore holes of Ambrosia beetles and other unidentified borer; c) long horn beetles; d) bark borer

IV. CONCLUSION

It is imperative that large scale management of Ambrosia and bark beetles was urgently needed to stop tree bean decline in Mizoram. Since weakened, dying or physiologically stressed trees are the preferred hosts of Ambrosia beetles and bark borer. Thus, maintaining tree health and vigour is an important step for reducing the risk of ambrosia beetle infestations and reducing tree density and maintaining proper spacing can strengthen the fitness of individual trees. Proper weeding, frequent watering and fertilization, pruning of infested trunk needs to be done. Stem injection and soil application of systemic insecticides will be useful to some extend and timing of foliar applications of insecticides needs to be properly worked out to increase the effectiveness as they were very difficult to control. Insecticides treated net may also be employed if applicable. Parasitoids and predators of these destructive pests need to be found out. Lastly, all the available management practices should be incorporated within the overall framework of integrated pest management to combat these notorious pests.

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Plant Water Consumption of SIIRT Pistachio using Blaney Cridlle and Penman - Monteith Methods

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Abstract— *Pistachio cultivation is carried out in many provinces of Turkey; however, it is mainly concentrated in the southeastern Anatolia Region due to the suitability of the climate. The pistachio is intensively grown in Gaziantep and Şanlıurfa, Adıyaman, Mardin and Siirt provinces of southeastern Anatolia, and has a significant impact on the economy of the region. Local varieties are mostly grown in Gaziantep and Şanlıurfa provinces, while Siirt variety is mainly grown in Siirt region. The continental climate of the Siirt province causes the summer to be extremely hot and the precipitation regime to be irregular. Therefore, irrigation in the region along with other cultural practices is important in pistachio cultivation. The increase in population and development of industrialization caused a significant decrease in natural resources; therefore, water resources have to be used as most economical and efficient way as possible. Sustainable use of limited water resources and continuous, high-level benefit from water can only be achieved by determining the amount of crop water consumption in accordance with the climatic conditions, and by creating appropriate irrigation programs considering the crop growing periods. This study was carried out to calculate the crop water consumption for Siirt Pistachio plant using modified Blaney Criddle and modified Penman–Monteith methods.*

Keywords— Siirt pistachio, Irrigation, Plant water consumption.

I. INTRODUCTION

Pistachio (*Pistacia vera* L.), which is grown in the Near East, Mediterranean Region, western regions of Asia and United States of America, is mainly grown in Gaziantep and Şanlıurfa provinces of Turkey. The number of provinces where pistachios are grown is 44 which are located in Southeast Anatolia, Mediterranean, Aegean and Central Anatolia Regions of Turkey, however, the highest production takes place in Gaziantep, Kahramanmaraş, Adıyaman, Şanlıurfa, Mardin, Kilis, Diyarbakır and Siirt provinces. There are many pistachio varieties in Turkey, and Siirt pistachio (Figure 1) has an important share among the varieties grown in Turkey. Domestic varieties are grown mostly in Gaziantep and Şanlıurfa provinces, while Siirt variety adapted to the region is grown in Siirt region. The Siirt pistachios grown in Siirt and Şanlıurfa are preferred because of their large grains and high crack rate. The pistachio is highly drought tolerant plant and can be grown in marginal areas where precipitation is very low (150 mm annual precipitation). However, irrigation water is needed at every stage of agricultural activity and pistachio varieties (Uzun, Siirt, Kırmızı, Halebi, etc.) also need irrigation.

Some researchers indicated that water stress may cause low yield and periodicity, which are among the most important problems of pistachio cultivation (Kanber et al., 1993). The reports revealed that irrigation has a positive effect on yield by reducing water stress. In addition, irrigation improved product quality and has the effect of reducing periodicity. Therefore, the irrigation is a prerequisite for optimum yield in pistachio cultivation. Similarly, Arpacı et al. (1995) stated that Siirt pistachio yields better under irrigated conditions than rainfed conditions, and therefore, Siirt pistachio cultivation should be carried out under irrigated conditions. The purpose of this study was to determine the crop water consumption of Siirt pistachio by modifying the Siirt province climate data to the Blaney Criddle equation.

FIGURE 1: SIIRT Pistachio Orchard

II. MATERIAL AND METHOD

The continental climate with cold and rainy winters and hot and dry summers is dominant in the Siirt region. The average temperature is 26 ºC in summer and 2.7 ºC in winter. The highest annual relative humidity is 70.2% in January and 26.9% in August. The annual average relative humidity is 50.41%. Total annual precipitation is 669.2 mm, and the monthly precipitation varies between 103.6 mm and 1.3 mm (Anonymous 2020). The soils of the experimental area are classified as brown forest soil (Dengiz et al., 2013). The soil has a clay texture, low electrical conductivity and the lime content does not pose a problem for plant growth. In addition, the soil has low phosphorus, high potassium and moderate organic matter content. Land use capability map of Siirt province is given in Figure 2.

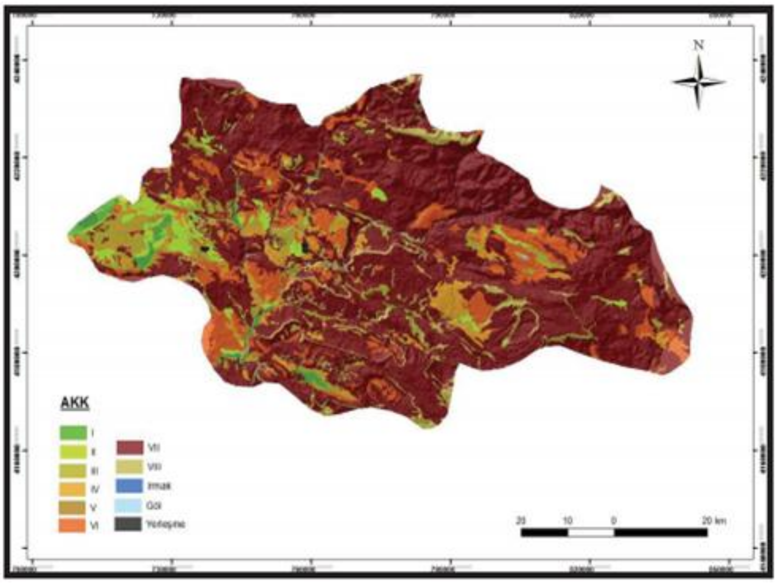

FIGURE 2: Land use capability map of Siirt province.

2.1 Plant water consumption

The concept of 'Evapotranspiration' (ET) in many national and international sources is defined as 'Crop Water Consumption' in Turkish. The ET refers to evaporation from soil and plant. In general, evaporation refers to the movement of liquid water from an environment to the atmosphere by evaporation. Evaporation of water from any surfaces, especially soil and open water surfaces, is called 'evaporation'. The energy must penetrate the water to start the evaporation. The source of energy in nature is mostly solar radiation and air temperature. Evaporated water moves through vapor pressure difference between the surface in question and the atmosphere surrounding this surface. The atmosphere surrounding the surface where evaporation occurs becomes saturated with water and the saturated air does not move, evaporation stops. At this stage, air movement occurs with winds. Evaporation occurs entirely based on solar radiation, air temperature, humidity and wind speed (Bayramoğlu 2013). The amount of shading the soil surface by the above-ground plant parts and the water content at the depth of the soil exposed to evaporation significantly affect the amount of evaporation from soil surface.

2.2 Determining Plant Water Consumption

Crop water consumption is both directly measured and estimated using climate data. The direct measurement methods may provide reliable results; however, they are quite expensive and time consuming. Therefore, the direct measurement of crop water consumption is only carried out to calibrate the estimation equations using the climate data and to obtain the local crop coefficients. Therefore, in practice, crop water consumption values are commonly determined using estimation equations based on climate data.

Many equations have been developed to estimate crop water consumption using climate data. Some of the equations developed by using several climatic factors are easy to solve, and can give accurate results for long periods. Others developed using many climatic factors affecting plant water consumption are rather complex equations, and give accurate results even for short periods.

The common way of estimating the crop water consumption values is to first define potential crop water consumption in which considering only climatic factors and develop empirical equations that can be used. Then, the potential crop water consumption values are corrected using the crop coefficients, which are the function of crop type and crop development stages (Güngör et al., 2004).

$$ET=Kc*ETp \tag{1}$$

In the equation; ET is the crop water consumption (mm day^{-1}), Kc is crop coefficient, ETp is the potential crop water consumption (mm day^{-1}).

Standard definition of potential crop water consumption has not yet been made, causing some confusion in the interpretation. Therefore, the concept of "benchmark crop water consumption" has been widely used recently instead of potential crop water consumption. Initially, a benchmark crop with certain conditions is determined and empirical equations are developed that can be used to estimate the water consumption of this crop. Then, the equations are corrected with the crop coefficients, which are a function of the crop species and crop growth stage, so that these equations can be used in the estimation of water consumption for other crops.

$$ET=Kc*ET_o \tag{2}$$

In the equation; ET is the crop water consumption (mm day^{-1}), Kc is crop coefficient, ETo is benchmark crop water consumption (mm day^{-1}).

Meadow plants, sesame, alfalfa and similar plants are used as benchmark plants. The benchmark of crop water consumption for meadow plants is defined as the water consumption in a large area covered with 8-10 cm high, same height, effectively growing, fully covering the area, adequately irrigated meadow plants.

2.2.1 Blaney-Criddle Method

The climate data used in this method are average temperature, daylight hours, minimum relative humidity and average daytime wind speed. The method gives rather rough results due to the use of only a few climatic factors. Therefore, the method is used for estimation of crop water consumption for at least monthly periods. The Blaney-Criddle equations used in estimating the benchmark crop water consumption for a given month are given below. The schematic representation of the Blaney-Criddle method.

ET_o=cf

f=p(0.46t+8)

In the equations; ETo daily average water consumption (mm day^{-1}), c is the correction factor, f is daily climate factor (mm day^{-1}), p is the ratio of mean daily daylight hours to annual daylight hours, and t is average daily temperature (°C).

First, the f values are calculated, and the ET_o values are taken directly from the relevant graphs. Average daytime wind speed is the value measured at 2 m height, and if there are no daytime average wind speed values, then 24-hour average wind speed values are obtained by multiplying by 1.33. The p value in the equation is taken from the relevant Tables according to the latitude of the region.

2.2.2 Penman-Monteith

Penman developed an equation for evaporation from the open water surface in 1948 using records of climate data (insolation, temperature, humidity, pressure, and wind speed). This method was further developed by Monteith in 1976 by adding aerodynamic and surface resistance factors for plants. In 1990, various experts came together and FAO developed the Penman-Monteith method. Although this method has different names in different countries, it is widely used as FAO56-PM with the concept of "reference crop water consumption" instead of potential water consumption ((Koç & Güner, 2005); (İlhan & Utku, 1998); Allen et al., 1998). In the Penman-Monteith Method, the water consumption of the plants (ETc) is determined following the reference crop water consumption is corrected with the single crop coefficient (kc) or the double crop coefficient (ke + kcb). In addition, the change in crop water consumption due to various stresses caused by drought, salinity, disease and other factors can also be determined by using the stress coefficient (ks) (Yürekli, 2010; Allen et al., 1998).

2.2.2.1 Penman-Monteith Method for Benchmark Crop Water Consumption

In this method, benchmark crop water consumption can be calculated using Equation 1.3, as follows;

$$ET = \frac{\delta}{\delta+\gamma^*}(R_n - G)\frac{1}{\lambda} + \frac{\gamma}{\delta+\gamma^*}\frac{900}{T+275}u_2(e_a - e_d) \quad (3)$$

The equations used in the calculation of some terms in Equation 3 are given below.

$$\delta = \frac{4098\, e_a}{(T+237.3)^2} \quad (4)$$

$$\lambda = 2.501 - 2.361 \times 10^{-3}T \quad (5)$$

$$\gamma = 0.0016286\frac{P}{\lambda} \quad (6)$$

$$\gamma^* = \gamma(1 + 0.34u_2) \quad (7)$$

$$R_n = R_{n_s} - R_{nl} \quad (8)$$

$$R_{n_s} = 0{,}75R_s \quad (9)$$

$$R_{n_l} = 2{,}451 f(T) f(e_d) f\left(\frac{n}{N}\right) \quad (10)$$

$$R_5 = \left(0.25 + 0.50\frac{n}{N}\right)R_a \quad (11)$$

$$e_d = e_a\frac{RH}{100} \quad (12)$$

$$u_2 = u_z\left(\frac{2}{z}\right)^{0.2} \quad (13)$$

In the equation;

ET is the reference plant water consumption, mm/day, δis the slope of wapor pressure line (kPa/°C)

γ^* *is the modified psicometric constant* ($kPa/°C$)

γ *is the psicometric constant* ($kPa/°C$)

$P\ is\ the\ atmospheric\ pressure\ (kPa)$

$R_n\ is\ the\ net\ radiation\ from\ plant\ surface\ (\frac{\frac{MJ}{M2}}{day})$

$R_a is\ the\ Radiation\ reaching\ the\ outer\ surface\ of\ the\ atmosphere\ (\frac{MJ}{M2}/day)$

$R_s\ is\ the\ short\ wave\ radiation\ reaching\ earth\ (\frac{MJ}{M2}/day)$

$R_{n_s}\ is\ the\ short\ wave\ net\ radiation\ (\frac{MJ}{M2}/day)$

$R_{n_l}\ is\ the\ long\ wave\ net\ radiation\ (\frac{MJ}{M2}/day)$

$f(T)\ is\ the\ temperature\ function$

$T = Sıcaklık, °\ C$

$f(e_d)\ is\ the\ vapor\ pressure\ function$

$e_d\ is\ the\ actual\ vapor\ pressure\ at\ average\ air\ temperature\ (kPa)$

$e_a is\ the\ saturated\ vapor\ pressure\ at\ average\ air\ temperature\ (kPa)$

$f(^n/_N)\ is\ the\ insolation\ rate\ function$

n is the insulation period (h)

N is the possible maximum insulation period (h)

G is the heat flow in soil (MJ/m^2/day) (Soil temperature does not change much in successive period; therefore, mean soil temperature can be neglected)

$\lambda\ is\ the\ latent\ heat\ of\ evaporation, \frac{MJ}{kg} \left(mean\ value\ of\ 2.45 \frac{MJ}{kg}\ can\ be\ used\right)$

$u_2\ is\ the\ wind\ speed\ at\ 2\ m\ height, m/s$

$u_z\ is\ the\ wind\ speed\ measured\ at\ z\ m\ height, \frac{m}{s}$

z is the height that wind speed measured, m

(In Turkey, meteorological bulletins usually give wind speed values measured at 10 m height), and RH is the average relative humidity (%).

In terms of pressure units;

1 mb = 0.1 kPa

and in terms of the radiation unit;

1 cal/cm^2/day =0.041868 MJ/m^2/day = 0.01706 mm/day.

2.3 General Climate Characteristics of Siirt Province

The region is under the influence of dry and hot tropical air masses settled in the low pressure center of Basra in the summer. The highest daily temperature rises above 40 degrees. The dry and hot winds called "samyeli", formed by the expansion of the Basra low pressure center towards Anatolia, cause both excessive evaporation and dust storms. In addition, sometimes dusty and polluted air coming from the Syrian and Arabian deserts cover the Siirt region. In the winter season, the region is under the influence of the precipitation fronts coming from the Central Mediterranean region and the precipitation continues until April (Atalay ve Mortan 2003).

The annual average temperature between 1938 and 2019 was 16.1 °C. The average temperature, which may be at the lowest level with the effect of external factors affecting Turkey during the winter months, start to rise rapidly as of March and rise above 25°C in May and June (Table 1). The average temperature in summer (June, July and August) is 26°C, which does not fall below 2.7°C in winter (December, January and February). The average temperature in January, the coldest month, is 2.7°C and 30.5°C in July, the hottest month, with an average temperature difference of 27.8°C. Another reason for defining a continental climate in the area where the experiment was conducted is the high temperature difference between the seasons. The average temperature in Siirt Center is 28.8°C in the summer months, while the average temperature in the winter months is 3.8°C. Temperature values in the experimental area are higher than in many regions of our country. There are several factors affecting the temperature variations, of which the latitude is an important factor. The area where the experiment was conducted is located in the south of Turkey. The experimental area receives the sun rays at steeper angles than many other regions of Turkey. In addition, the landforms are effective on the temperature values of the area where the experiment was conducted.

The basin where the experiment was conducted is located on the outskirts of the Southeastern Taurus Mountains of the Taurus mountain range. Therefore, the effect of cold air coming from the north is not frequently observed. In the south, there are no obstacles to prevent the effects of hot weather. Another important factor affecting the temperature in the region is the continental climate dominating our country in general. The air temperature in the regions with continental climate rises very rapidly and become very hot. Therefore, summers are very hot and winters are very cold in the region. Low altitude and cloudiness are other factors affecting the warming in the region. The distribution of precipitation also varies according to the seasons. The season with the highest precipitation is spring with 40%, followed by winter with 38%. Precipitation in winter and spring constitutes 78% of the total. In autumn, 20% precipitation occurs, and the least precipitation is in summer, with a rate of 2%. Snowfall is low in the region and generally occurs in December, January and February. Winter is the season when both snowfall occurs and the number of days covered with snow is the highest.

The average wind speed slightly changes during a year and it was in the region between 1938 and 2019 is1.2 m/sec The highest average wind speed is 0.6 m/sec in March, April, May, June, July, August and September, and the lowest value is 0.3 m/sec in November, December and January. The highest wind speed occurs in October (5.8 m/sec) and the lowest wind speed (2.4 m/sec) in December and March. The highest number of wind blows occurs in the spring with 26% (42,487), followed by summer and winter with 25% and autumn with 24%.

TABLE 1
LONG TERM METEOROLOGICAL DATA FOR SIIRT PROVINCE (1938-2019).

Parameter	Max. Temp. (°C)	Min. Temp. (°C)	Mean Relative Humidity (%)	Mean Total Precipitation (mm)	Maximum Precipitation (mm)	Mean Evaporation (mm)	Mean Insulation duration (hour)
Duration of the records (Year)	**79**	**79**	**78**	**78**	**79**	**79**	**57**
January	19.7	-19.3	71.9	34.6	53.4	12.0	3.6
February	20.6	-16.5	67.1	29.4	53.2		4.4
March	28.5	-13.3	62.0	24.1	63.0	33.0	5.4
April	32.9	-4.1	58.0	22.4	71.4	84.0	6.5
May	36.2	2.0	50.7	21.2	68.1	186	9.0
June	40.2	8.2	34.6	15.5	16.7	284.8	11.7
July	44.4	13.1	27.4	13.5	22.2	368.0	12.2
August	14.4	46.0	26.4	13.3	12.2	351.8	11.4
September	39.9	8.5	31.2	14.4	37.5	254.3	9.9
October	36.6	0.3	46.7	49.7	70.8	137.6	7.2
November	25.8	-14.1	62.4	82.5	102.9	53.0	5.2
December	24.3	-14.6	70.6	94.5	71.8	13.1	3.6
Annual	46	-19.3	50.8	719.8	102.9	1753.6	7.5

In addition, the latitude degree, average temperature, wind speed, average relative humidity, duration of insulation and atmospheric (actual) pressure values of the region and the period for the crop water consumption will be calculated were taken from the 15th Regional Meteorology Directorate station in Siirt province.

2.4 Climate Data of Siirt Province between 2019 and 2021

Average wind speed, relative humidity, temperature, atmospheric (actual) pressure and insulation duration data for June, July, August, September and October were prepared in Batman 15th Regional Meteorology Directorate and obtained from Siirt Meteorology Directorate.

2.4.1 Data of the Study (Blaney – Criddle)

- **Latitude of Siirt province:** 38° 42 '
- **Periods:** June, July, August, September and October in 2020
- **Mean temperature, T :** 28.0°C
- **Wind speed measured at 10 m height, u_{10} :** 1.2 m/s
- **Mean relative humidity, RH :** % 24.7
- **Insulation period, n :** 10 h 39 minutes

1) At the beginning f value is v-calculated.

The p value is equal to 0.301 based on the periods and the location (38° 42').

2) Insulation rate

n= 10 h 39 min. = 10 45 h

TABLE 2
THE HIGHEST POSSIBLE INSULATION PERIOD, N (h/day).

Latitude	Months							
	March	April	May	June	July	August	September	October
44	11.9	13.4	14.7	15.4	15.2	14.0	12.6	11.0
42	11.9	13.4	14.6	15.2	14.9	13.9	12.6	11.1
40	11.9	13.3	14.4	15.0	14.7	13.7	12.5	11.2
35	11.9	13.1	14.0	14.5	14.3	13.5	12.4	11.3
30	12.0	12.9	13.6	14.0	13.9	13.2	12.4	11.5

By interpolation;38° 42 ' latitude and

- For June N = 14.8 h
- For July N = 14.5 h
- For August N = 13.6 h
- For September N = 12.46 approximately 12.5 h and
- For October N = 11.24 approximately 11.2 h

13.32 h approximately 13.3h is used.

3) Equivalent of daily wind speed at 2 m height

$$\mu_2 = \mu_z \left(\frac{2}{z}\right)^{0.2} = 1.2\ m/s$$

4) Benchmark crop water consumption

RH_{min} = % 24.7, n/N = 0.79

When RH_{min} = % 24.7, n/N = 0.79 and u_z= 1.2 m/s.

$$f = p(\ 0.46t + 8\ )$$

= 0.301*(0.46*28 + 8) = 6.28 mm/day is calculated.

From this value, draw a perpendicular line to the 2nd line, and look at the left from the intersection, and ET_o is calculated as 7.9mm/day. Finally, reference daily crop water consumption is calculated as 7.9 mm.

When the average number of days for June, July, August, September and October is considered as 30 days, reference crop water consumption will beET_o= 30 * 7.9 = 237 mm/month.

2.4.2 Data of the study

- **Latitude of Siirt province:** 38° 42 '
- **Periods:** For June, July, August, September and October in 2020
- **Average temperature, T:** 28.0°C
- **Wind speed at 10 m height, u_{10} :** 1.2 m/s
- **Mean relative humidity, RH :** %24.7
- **Insulation period, n :** 10h 39 minutes
- **Atmospheric pressure (actual pressure),** P : 909 mb = 90.9 kPa

1) Saturated vapor pressure at average air temperature is determined.

TABLE 3
SATURATED VAPOR PRESSURE AT AVERAGE AIR TEMPERATURE, e_a

T, ∘ C **e_a, kPa**	1 0.66	2 0.71	3 0.76	4 0.81	5 0.87	6 0.93	7 1.00	8 1.07	9 1.15
T, ∘ C **e_a, kPa**	10 1.23	11 1.31	12 1.40	13 1.50	14 1.61	15 1.70	16 1.82	17 1.94	18 2.06
T, ∘ C **e_a, kPa**	19 2.20	20 2.34	21 2.49	22 2.64	23 2.81	24 2.98	25 3.17	26 3.36	27 3.57
T, ∘ C **e_a, kPa**	28 3.78	29 4.01	30 4.24	31 4.49	32 4.76	33 5.03	34 5.32	35 5.62	36 5.94

For T = 28.0 °C e_a **3.78 kPa**

1) Saturated vapor pressure at average air temperature is calculated.

$$e_d = e_a \frac{RH}{100} = 3.78 * \frac{24{,}7}{100} = \mathbf{0.9\ kPa}$$

2) The slope of the vapor pressure line is calculated.

$$\delta = \frac{4098 e_a}{(T+237.3)^2} = \frac{4098 * 3.78}{(28+237{,}3)^2} = \mathbf{0.220\ kPa/°C}$$

3) The psychrometric constant is calculated

$$\gamma = 0.0016286\frac{P}{\lambda} = 0.001 * \frac{90.9}{2{,}45} = \mathbf{0.0604}\ \boldsymbol{kPa}/°\mathbf{C}$$

4) Wind speed at 2m height;

$$u_2 = u_z\left(\frac{2}{z}\right)^{0.2} = \mathbf{1.2 m/s} \text{ is given.}$$

5) Modified psychrometric constant is calculated.

$$\gamma^* = \gamma(1 + 0.34u_2) =$$

$$0.0604 * (1 + 0.34 * 1.2) = \mathbf{0.085}\ \boldsymbol{kPa}/°\mathbf{C}$$

6) Maximum possible insulation duration is found.

By interpolation;

38° 42 ' latitude and

- For June N = 14.8 h
- For July N = 14.5 h
- For August N = 13.6 h
- For September N = 12.46 approximately 12.5 h and
- For October N = 11.24 approximately 11.2 h.

The average of these values is calculated: 13.32 h approximately 13.3 is used.

7) Insulation rate is calculated.

n = 10 h 39 minutes = 10.45 h

$$\frac{n}{N} = \frac{10.45}{13.3} = \mathbf{0.78}$$

8) Radiation reaching the outer surface of the atmosphere is found.

38° 42 ' latitude and

- For June Ra = 42.2 MJ/$m2/day$
- For July Ra =40.9 MJ/$m2/day$
- For August Ra = 37.5 MJ/$m2/day$
- For September Ra = 31.4 MJ/$m2/day$
- For October Ra = 24.5 MJ/$m2/day$

From here, the average is calculated as 35.3 MJ/$\boldsymbol{m2/day}$.

9) The short-wave radiation reaching the earth is calculated.

$$R_s = \left(0.25 + 0.50\frac{n}{N}\right)R_a$$

= (0.25+0.50*0.78)*35.3 = **22.6 MJ/$\boldsymbol{m2/day}$**

10) The short-wave net radiation is calculated.

$$R_{n_s} = 0{,}75R_s$$

= 0.75*22.6 = **16.9MJ/$\boldsymbol{m2/day}$**

11) Temperature function is found.

For T = 28°C f(T)= **16.3**

12) Vapor pressure function is found.

$$e_d = 0.8\ kPa\ için\ \mathrm{f}(e_d) = 0.22\ and$$

$$If\ e_d = 1.0\ kPa\ için\ \mathrm{f}(e_d) = 0.20, then\ interpolation\ is\ carried\ out,$$

For $e_d = 0.9\ kPa\ \mathrm{f}(e_d) is\ used\ as\ \mathbf{0.23}$.

13) The insulation rate is found.

For n/N =0.75 f(n/N) = 0.78,

for n/N =0.80 f(n/N) = 0.82, then

for n/N =0.78 f(n/N) = **0.80.**

14) Long wave net radiation is calculated.

$$R_{n_l} = 2{,}451 f(T) f(e_d) f\left(\frac{n}{N}\right)$$

= 2.451*16.3*0.23 = **9.19 MJ/$m2/day$**

15) Net radiation on plant is calculated.

$$R_n = R_{n_s} - R_{nl}$$

= 16.9- 9.19 = **7.71 MJ/$m2/day$**

16) Benchmark plant water consumption is calculated.

$$ET = \frac{\delta}{\delta+\gamma^*}(R_n - G)\frac{1}{\lambda} + \frac{\gamma}{\delta+\gamma^*}\frac{900}{T+275}u_2(e_a - e_d) =$$

$$\frac{0{,}220}{0{,}220+0{,}085} * (7.71 - 0) * \frac{1}{2.45} + \frac{0.0604}{0.220+0.085} * \frac{900}{28+275} * 1.2 * (3.78 - 0.9) = \mathbf{8.0\ mm/day}.$$

When the average number of days for June, July, August, September and October is considered as 30 days, reference crop water consumption will be ET_o= 30 * 8.00 = 240 mm/month.

III. RESULTS

The daily reference water consumption calculated using Blaney-Criddle estimation method is **7.9 mm,** and the daily crop water consumption calculated using Penman – Monteith method was **8.00 mm**. The monthly average (considering June, July, August, September, October) water consumption will be **237 and 240 mm/month,** respectively.

IV. CONCLUSION AND RECOMMENDATIONS

Climatic change, which has emerged as a serious threat due to global warming, leads to the depletion of limited water resources. Water is extensively used in agricultural production; therefore, determining the amount of long or short term plant water consumption realistically depending on the climate data is of great importance for irrigation projects. Therefore, one of the Blaney-Criddle and Penman-Monteith methods, which estimate crop water consumption using meteorological data, can be preferred. An appropriate irrigation program should be established according to the climatic conditions of each region to conserve water resources. For this purpose, the water consumption of crops grown needs be calculated and an irrigation program should be prepared, using the climatic data of the region.

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Impact of Different Concentrations of Municipal Wastewater on Rice Seed Germination and Seedling Performance

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Abstract— *The major challenge faced by developing countries is to produce adequate food for their growing human population under the shortage of fresh water for agricultural activities. Laboratory experiment was conducted in a completely randomized design using sand medium with municipal wastewater and MR253 rice seed with the aim to evaluate effects of municipal wastewater (treated and untreated) at different concentrations (0, 2.5, 5, 10, 25, 50 and 100%) on seed germination and seedling performance. Significant ($p<0.05$) difference was observed between untreated and treated municipal wastewater for seedling length (SL), root volume (RV), seedling vigour index (SVI) and root: shoot ratio (R:S) while no difference were observed between untreated and treated municipal wastewater for germination percentage (GP) and the seedling phytotoxicity. The concentrations of N, P, K, Ca, Mg, Zn, Fe, Cu and Mn were higher in the untreated municipal wastewater compare to treated municipal wastewater. Seeds imbibed with untreated municipal wastewater have high seed germination and seedling performance compare to treated municipal wastewater. Inhibitory effect on chlorophyll content was observed at concentration >50% of both untreated and treated municipal wastewater while promoting effects were observed at lower (<25%) concentrations. However, all the nutrient elements were negatively and highly correlated with quantity of different municipal wastewater concentration. The study showed that seeds imbibed with untreated municipal wastewater have high seed germination and seedling performance compare to treated municipal wastewater. Municipal wastewater of <50% concentration could be recommended as a good source of water and nutrients for rice seed germination without affecting seedling performance.*

Keywords— Municipal wastewater, Seed germination, Seedling Performance, Oryza sativa, nutrient uptake, Chlorophyll content, Correlation.

I. INTRODUCTION

Potential means to secure rice productivity is to ensure that the quality of the seeds for sowing is good. A good and quality seeds are free of weed seeds, seed-borne diseases, pathogens, insects, or other matters and it possess high germination, vigor, viability and seedling performance (Chhetri, 2009). On the other hand, water is a major factor on earth and top priority for the existence of human life and crop production. The global demand for water in agriculture will have to increase with rising human population, escalating incomes, and deviations in nutritional favorites. Growing demands for water by industrial, urban users will deepen competition (de Fraiture and Wichelns, 2010). The use of domestic wastewater for agricultural production is increasing, especially as domestic wastewaters are rich in plant nutrients and organic matters which are essential for plant growth and development (Dash, 2012). The practice of using municipal wastewater will help reduce the pressure on fresh water for watering or irrigation of agricultural activities. Crops irrigated with wastewater have the potential to give a higher yield as the water helps to reduce the need for chemical fertilizers (Haussain et al., 2002). The macro and micro-nutrients in the wastewater assist as a good basis of plant nutrients and the organic constituents furnish helpful soil conditioning properties (Singh and Agrawal, 2008). Generally, wastewater (treated and untreated) is extensively utilized in farming because it is a rich basis of nutrients and provides all the moisture vital for crop growth. Water is a vital factor in agriculture and it plays an influential role in the growth and development of plants. Therefore, actions must be taken to reinforce irrigation farming, safeguard the rural environment and support water resource sustainability with good water management (de Juan *et al.*, 1999). Meeting the trials of feeding the ever-rising human populace, proficient uses of water and land resources is extremely vital in crop production. As the demand of wastewater is increasing, this study was conducted to evaluate the impact of untreated and treated municipal wastewater on rice seed germination and seedlings performance.

II. MATERIAL AND METHOD

2.1 Municipal Wastewater Source

Municipal wastewater used for this study was collected from Indah Water konsortium Berhad, wastewater treatment plant in Kuala Lumpur, Malaysia. Standard procedure (APHA, 1998) was followed during the collection and analysis of the wastewater samples. Some physico-chemical characteristics of the municipal wastewater were analyzed in the Department of Crop Science, Universiti Putra Malaysia by using Auto-Analyzer (Lachat 8000 Series) to analyzed nitrogen, phosphorous and potassium. Iron, zinc, calcium, magnesium, manganese, copper were analyzed by using atomic absorbtion spectrometer (Perkin Elmer AAnalyst 400) while cadmium and lead were analyzed by using inductively coupled plasma (Perkin Elmer, Optima 8300) at the Department of Land Management, Universiti Putra Malaysia. The analysis for biological oxygen demand (mg/l), chemical oxygen demand (mg/l), ammonium (ml/g), nitrate (ml/g), total suspended solid (mg/l), pH, oil and grease (ml/g) were done by Indah Water Konsortium Berhad, wastewater treatment plant Kuala Lumpur, Malaysia.

2.2 Experimental Procedures

A rice variety, MR253 was planted on sand medium in the Seed Science Laboratory, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia. The rice seeds were watered with untreated (raw) and treated (processed) municipal wastewater samples.

2.3 Seed Preparation and Imbibition Treatment

Rice variety MR253 was sterilized with 70% of chlorox solution for fifteen minutes to remove microbes from the seeds. Double sterilized water was used to repeatedly wash the seeds. The seeds were then imbibed in different concentrations of untreated (raw) and treated (processed) municipal wastewaters and distilled water for six hours before planting. One Hundred healthy treated rice seeds were put in plastic germination boxes of 2.5kg of sterilized sands and the sands were moisturized with 500 mL of different concentration of wastewater before planting the seeds. Two hundred and fifty milli-liter (250 mL) of different concentrations of wastewater and distilled water were used for watering.

2.4 Experimental Design and Treatments

The experiments were conducted as factorial experiments using the completely randomized design with three replications with; untreated and treated municipal wastewater, seven concentrations of untreated and treated municipal wastewater diluted with distilled water (0, 2.5%, 5%, 10%, 25%, 50% and 100%) and one Malaysian Rice varieties (MR253). The boxes were placed in the laboratory at 25ºC under constant light. The germination test was conducted according to The International Seed Testing Association procedures (ISTA, 1999) and it was carried out for a period of two weeks.

2.5 Procedure for nutrient analysis

Determination of nutrients was carried out using the modified method of Wolf (1982). The seedlings root and leaf samples were placed into envelop and then dried in the oven at 70ºC for 48 hours. The dried plant tissues were grinded and 0.25 g was used for the digestion. For the digestion process, the samples were transferred into clean digestion flasks and 5mL of concentrated H_2SO_4 was added to each flask for 2 hours. Thereafter, the flasks were heated for 45 minutes at 285ºC and 2ml of 50% (H_2O_2) was added to complete the process. The process was repeated several times until the samples became clear. The flasks were removed from digestion plate, cooled at room temperature and then diluted to make up to 100mL volume with distilled water. Then the macronutrients N, P and K were determined in the solution by using Auto-Analyzer (Lachat 8000 Series) while Ca, Mg and micronutrients Zn, Fe, Cu, Mn were determined in the solution by using Atomic Absorption Spectrometer (Perkin Elmer model 3110). Nutrients uptake by seedlings were determined as a function of the rice dry biomass production.

2.6 Chlorophyll Content

The chlorophyll content was measured according to the procedure described by Porra et al. (1989). The amount of 0.2 g of fresh leaf was homogenized in 80% acetone for 2 minutes and then was centrifuged at 2500 rpm for 20 minutes and supernatant was extracted. About 3.5 mL of samples were pipetted into microfuge and the chlorophyll content was measured by using scanning spectrophotometer (UV3101 PC). The samples were read at wavelength of 663 nm and 646 nm. The formulae of Lichtenthaler and Wellburn (1983) were used to calculate chlorophyll a and chlorophyll b contents.

2.7 Data Collection

Data was collected on the following parameters: germination percentage, seedling length, root volume, seedling vigour index, root:shoot ratio and seedling phytotoxicity.

According to the procedure of Abdul Baki and Anderson (1973).

Seedling Vigour Index (SVI) =Germination (%) x Seedling length (cm)

$$\text{Root: shoot ratio} = \frac{\text{Dry weight for root}}{\text{Dry weight for shoot}}$$

According to the procedure of Chou and Lin (1976).

$$\text{Seedling phytotoxicit} = \frac{\text{Radical length of control} - \text{Radical lenght of test}}{\text{Radical length of control}} \times 100$$

Root volume was measured by using the root scanner and analysis machine (Analyzer WinMagRhizo, Epson Expression 1680).

2.8 Statistical Analysis

The SAS statistical software (9.4 versions) was used to analyze the data including analysis of variance (ANOVA). Treatments means were compared using least significance difference (LSD) at $P<0.05$.

2.9 Toxicity Threshold in Crops

The municipal wastewater was checked for trace element, toxicity hazards, particularly when trace element contaminations were suspected. The maximum levels of trace element recommended for crop production is shown in Table 1 below.

TABLE 1
RECOMMENDED MAXIMUM LEVELS OF TRACE ELEMENTS FOR CROP PRODUCTION (FAO, 1985)

	Element	Recommended maximum concentration (mg/L)	Remarks
Cd	(cadmium)	0.01	Toxic to beans, beets and turnips at concentrations as low as 0.1 mg/l in nutrient solutions. Conservative limits recommended due to its potential for accumulation in plants and soils to concentrations that may be harmful to humans.
Cu	(copper)	0.20	Toxic to a number of plants at 0.1 to 1.0 mg/l in nutrient solutions.
Fe	(iron)	5.0	Not toxic to plants in aerated soils, but can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum. Overhead sprinkling may result in unsightly deposits on plants, equipment and buildings.
Mn	(manganese)	0.20	Toxic to a number of crops at few-tenths to a few mg/l, but usually only in acid soils.
Pb	(lead)	5.0	Can inhibit plant cell growth at very high concentrations.
Zn	(zinc)	2.0	Toxic to many plants at widely varying concentrations; reduced toxicity at pH > 6.0 and in fine textured or organic soils.

III. RESULT AND DISCUSSION

3.1 Municipal wastewater characteristics

The analyses of both untreated and treated municipal wastewaters are shown in Table 2. The municipal wastewater had differences their chemical and physical characteristics. Municipal wastewater for both untreated and treated municipal wastewater was slightly acidic and alkaline in nature with pH range between 6.8 and 7.1. The characteristics of the wastewater used for watering showed that the presence of high amount of nutrients in untreated municipal wastewater than treated municipal wastewater.

TABLE 2
CHEMICAL AND PHYSICAL CHARACTERISTICS OF UNTREATED AND TREATED MUNICIPAL WASTEWATER

Parameters	Untreated wastewater	Treated wastewater
Color	Dark black	Clear
Nitrogen (mg/L)	24.2	14.5
Phosphorous (mg/L)	2.99	0.33
Potassium (mg/L)	5.45	0.35
✚ Iron (mg/L)	2.11	0.14
✚ Zinc (mg/L)	1.06	0.27
✚ Calcium (mg/L)	20.65	16.51
✚ Magnesium (mg/L)	3.86	1.59
✚ Manganese (mg/L)	3.02	1.87
✚ Copper (mg/L)	3.25	1.32
✚ Cadmium (mg/L)	0.02	0.01
✚ Lead (mg/L)	nil	nil
*Biological Oxygen Demand (mg/L)	247	6.9
*Chemical Oxygen Demand (mg/L)	436	32.2
*Ammonium (mg/L)	30	22
*Nitrate (mg/L)	3.2	1
*Total suspended solid (mg/L)	280	8.8
*Oil and grease (mg/L)	3.5	1.5
*pH	6.8	7.1

Sources: Analytical Lab, Department of Crop Science, Universiti Putra Malaysia
✚ *Analytical Lab, Land Management, Faculty of Agriculture, Universiti Putra Malaysia.*
**Indah Water Konsortium Berhad, wastewater treatment plant Kuala Lumpur, Malaysia.*

3.2 Seed Germination

Different concentrations of untreated and treated municipal wastewater have significant difference ($p<0.05$) on rice seed germination. Germination percentage increased with increase in concentration up to 25% and thereafter decreased gradually for both untreated and treated municipal wastewater (Table 3). Maximum germination percentage was recorded at 25% concentration with 95.1% and 92.5% germination for untreated and treated wastewater, respectively. Lower wastewater concentration had promoting effect on seed germination while higher wastewater had reducing effect. Similar result on rice in previous studies was recorded when the seeds were imbibed in municipal wastewater (Gassama *et al*., 2015). Dash (2012) studied the impact of domestic wastewater on seed germination and physiological parameters of rice and wheat, and observed that at higher concentration, percentage germination of seeds were reduced. Similar result was also observed by Saravanamoorthy and Kumari (2007) in peanut. Furthermore, Singh et al., (2007) observed significant decrease in the percentage germination and seedling vigour of rice and wheat with an increase in spent wash concentration. The decrease may be due to the adverse effect of high toxicity of the wastewater at higher concentration (Ramana et al., 2002; Yousaf et al., 2010).

TABLE 3
MEAN COMPARISON OF DIFFERENT CONCENTRATIONS OF TREATED AND UNTREATED MUNICIPAL WASTEWATER ON PERCENTAGE GERMINATION FOR MR253 RICE SEEDLINGS

	CONC (%)	UTWW	TWW
MR253	0	84.0a	86.2b
	2.5	86.3b	85.0b
	5	87.6b	87.0b
	10	89.1b	85.3b
	25	95.1a	92.5a
	50	79.3c	82.3c
	100	76.6c	80.1c

***Means within columns with common letters are not significantly (P>0.05) different**
UTWW = Untreated wastewater
TWW = Treated wastewater
CONC = Concentrations

3.3 Seedling Length

Different concentrations of untreated and treated municipal wastewater on seedling length for variety MR253 was significantly different at p<0.05. Results showed that seedling length increased with increase in concentration of untreated wastewater up to 5% concentration followed by decline. On the other hand, seedling length increased with increase in concentration of treated wastewater up to 50% and further increase in the wastewater concentration, decrease in seedling length was observed (Table 4). Dash (2012) reported significant declined in seedling length when seeds were treated with sewage at higher than 75%. However, seed treated at 25%-50% wastewater concentration had increased seedling lengths in both rice and wheat. Nawaz *et al*. (2006) reported that seedling length of soybean in industry effluent decreased with increase in concentration of the effluent while in marble industry effluent, increase in seedling length was recorded. Dhanam (2009) observed that 100% concentration of dairy effluent inhibited rice seedling growth and suggested that this may be due to osmotic pressure caused by high effluent dose. The decrease in seedling length at higher concentration can be due to the presence of high amount of heavy metals at higher concentration in the industrial wastewater they were using. Increased in seedling length at lower concentrations of effluent might be due to the presence of nitrates and sulphates which stimulate the protein production and other organic molecules required for plant growth (Yousaf *et al.*, 2010).

TABLE 4
MEAN COMPARISON OF DIFFERENT CONCENTRATIONS OF TREATED AND UNTREATED MUNICIPAL WASTEWATER ON SEEDLING LENGTH FOR MR253 RICE SEEDLINGS

	CONC	UTWW	TWW
MR253	0	58.16c	47.88b
	2.5	74.78b	47.36b
	5	81.70a	49.79b
	10	73.29b	52.53a
	25	60.41c	53.23a
	50	56.60c	54.27a
	100	58.96c	46.95b

***Means within columns with common letters are not significantly (P>0.05) different**
UTWW = Untreated wastewater
TWW = Treated wastewater
CONC = Concentrations

3.4 Root Volume

Different levels of wastewater concentrations were significantly (p<0.05) different on root volume for MR253 rice seedlings. Results showed that root volume increased with increase in concentration of up to 25% followed by a decline in MR253 (Fig:

1). The result showed that lower concentration has stimulating effect on root volume while higher concentration has deleterious effect on root volume. Maximum root volume 0.25 cm^3 was recorded when watered with untreated municipal wastewater compared to treated municipal wastewater 0.19 cm^3 (Fig: 2). Similar result was also observed in previous study by Gassama *et al.*, (2015) in rice. Rehman *et al.* (2009) observed significant reduction in root volume of three vegetables crops when exposed to 100% concentration of effluent while increased was observed at lower effluent concentration. Dhanam (2009) observed that higher concentration of dairy effluent inhibited rice seedling growth and suggested that this may be due to the water toxicity caused by high effluent dose.

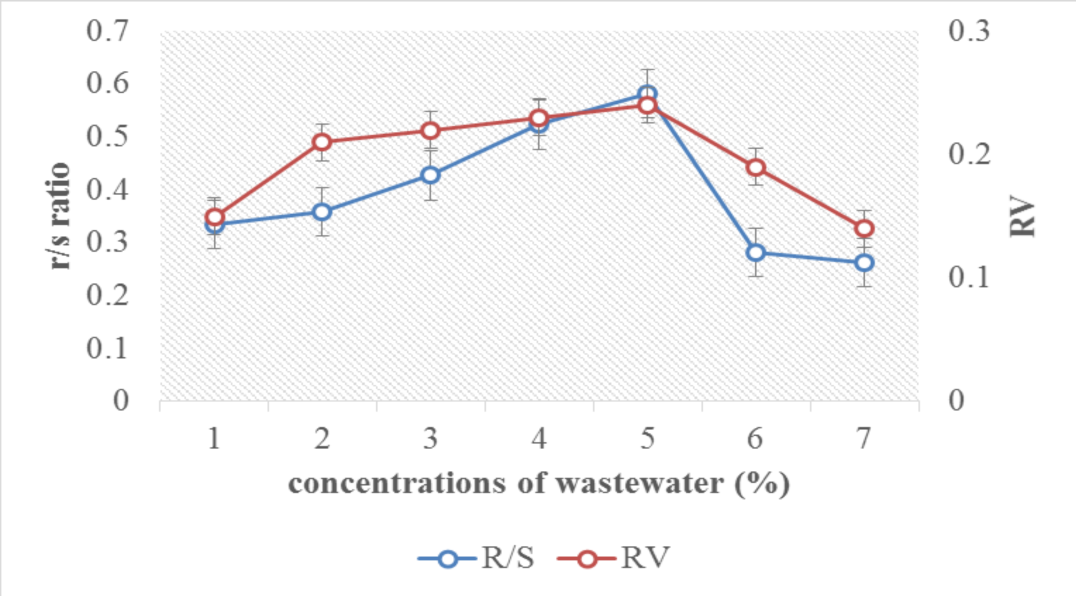

FIGURE 1: Effect of different concentrations of municipal wastewaters on root volume and root/shoot ratio for MR253 rice variety.

r/s= root/shoot ratio; RV= root volume;

Vertical bars on line represent error bars with percentage (±)

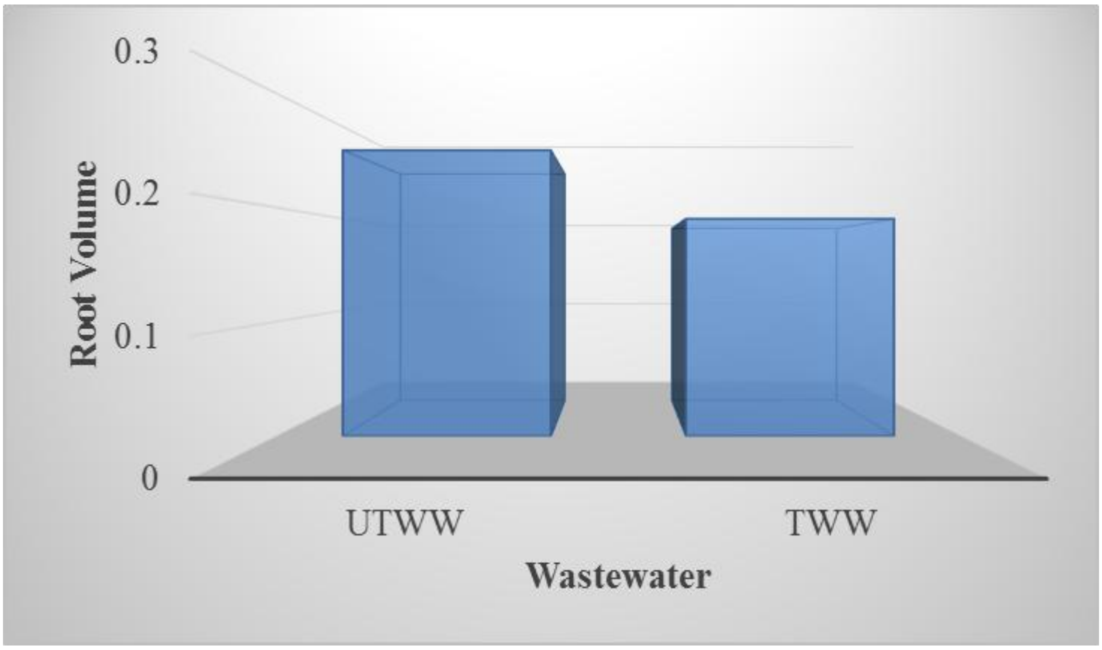

FIGURE 2: Effect of treated and untreated municipal wastewater on root volume (RV) for MR253 rice seeds.

3.5 Root/Shot Ratio

Result showed that MR253 rice seedlings watered with different concentration of municipal wastewater showed significant ($p<0.05$) difference for root:shoot ratio. Rice seedlings irrigated with distilled water (control) gave normal root/shoot ratio and any changes from this normal level either up or down would be an indication of a change in the overall health of the crop. Maximum root/shoot ratio was recorded at 25% concentration for MR253 rice seedlings. Furthermore, increasing the concentrations to 50% and 100% of the wastewaters saw reduction in the root:shoot ratio MR 253 rice seedlings (Fig: 1). Increase in root/shoot ratio was observed at lower concentration of municipal wastewater for MR253 rice seeds while decreased ratio was observed at higher concentrations of the wastewaters. An increase in root:shoot ratio is an indication of a healthier plant while decrease in root:shoot ratio is an indication of deterioration in the health of the crops. The direct use of the raw wastewater resulted in decreased benefits whereas diluted concentrations noticed higher seed quality parameters because of lesser toxicity and better utilization of plant nutrients by the seedlings (Manunatha, 2008).

3.6 Seedling Vigour Index

The data pertaining to the seedling vigor index for MR253 rice seedlings as influenced by different concentrations of municipal wastewater was significantly ($p<0.05$) (Fig. 3). It was observed that the gradual but steady increase in various concentration levels of municipal wastewaters from 0% to 25% increased the seedling vigor index. Further increase in the municipal wastewater concentration from 50% to 100% saw significant decline in seedling vigor index for seeds. However, seeds watered with 25% concentration of municipal wastewater were the most vigorous seedlings with SVI of 6850 and seeds watered with 100% concentration of wastewater had the lowest seedling vigor index of 4533. Similar result has been recorded in previous studies when rice seeds were imbibed in municipal wastewater (Gassama *et al*., 2015). The seeds that gave the highest seedling vigor index for MR253 rice seeds are considered to be most vigorous (Abdul-Baki and Anderson, 1973). When the wastewaters are diluted in disparate concentrations, the toxicity of the wastewater's constitution goes on weakening and at an optimum concentrations larger utilization of nutrients takes place (Manunatha, 2008).

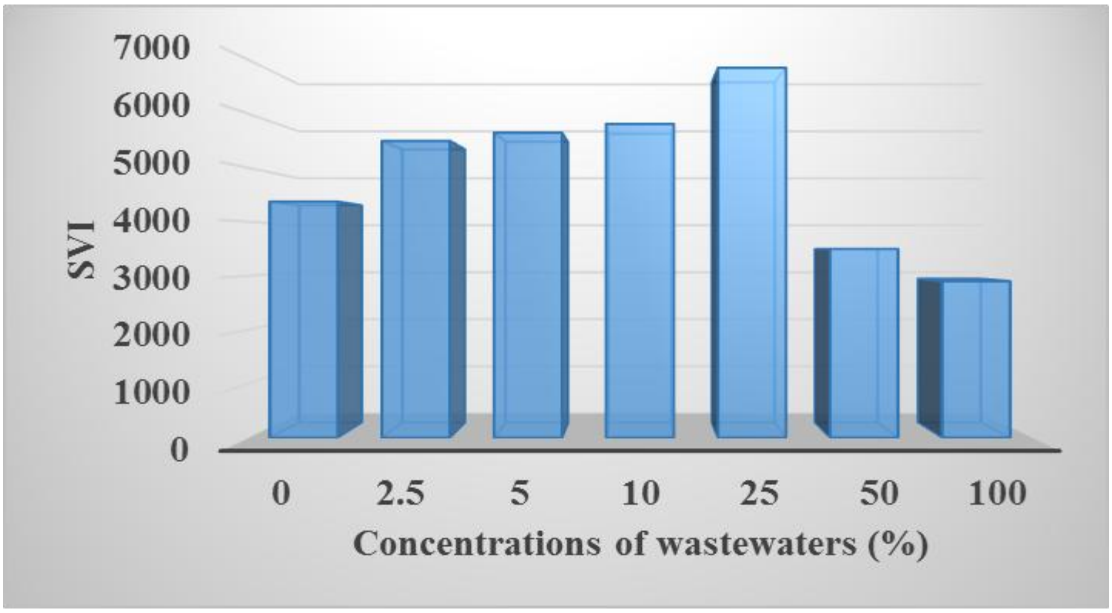

FIGURE 3: Effect of different concentrations of municipal wastewaters on seedling vigour index for MR253 rice variety.

3.7 Seedling Phytotoxicity Index

Data showed significant ($p<0.05$) difference on phytotoxicity for MR253 rice seedlings when imbibed in different concentrations of treated and untreated municipal wastewater. Lower wastewater concentrations (<25%) had lower phytotoxicity on the rice germination process that then gave better response of the MR253 rice seeds to the wastewater. However, further increase in the concentrations of the wastewater from 50%-100% resulted in higher phytotoxicity on the rice germination process that led to retarded growth for MR253 rice seeds (Fig: 4). The wastewater at lower concentrations had less toxicity that was unable to hinder the growth and development of the crops while at higher concentrations its toxicity was high enough to reduce growth and development of the crops. Higher wastewater concentrations have high phytotoxicity. Higher concentration of wastewater effluent decreases activities of dehydrogenase (Murkumar and Chavan, 1987) and acid phosphatase (De Leo and Sacher, 1970) which are important enzymes during early germination process and also involved in mobilization of nutrient reserves (Flinn and Smith, 1967). The low amount of oxygen in dissolved form due to high concentration of dissolved solids in the effluent reduces the energy supply through anaerobic respiration causing retardation of growth and development of seedling (Saxena *et al.*, 1986). The enhancement of seed quality by the lower concentrations of the wastewater was due to the presence of low toxic activities in the wastewater.

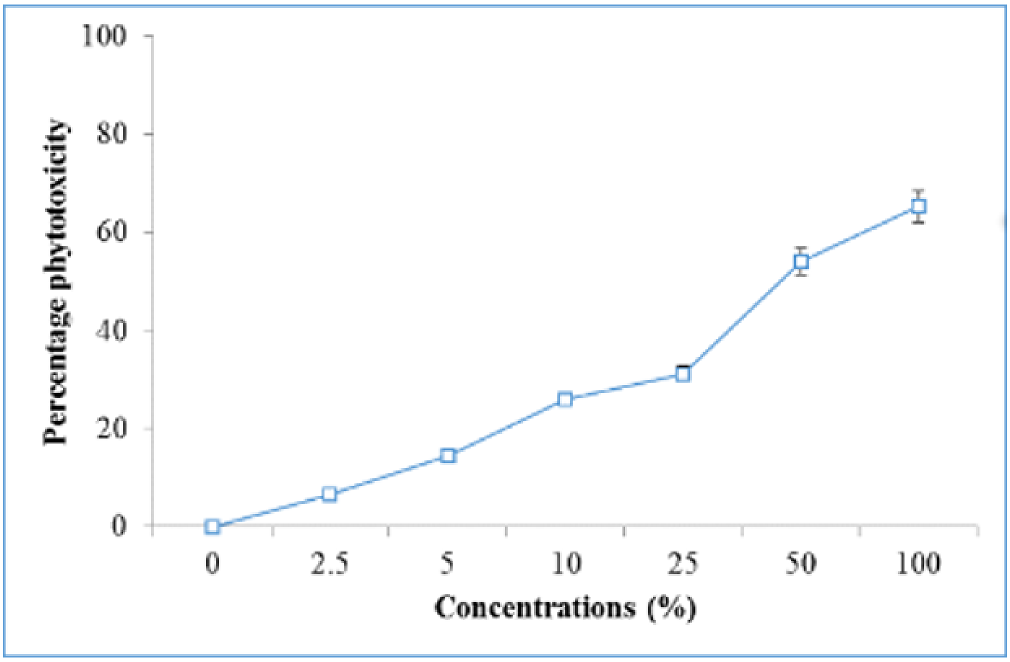

FIGURE 4: Effect of different concentrations of municipal wastewaters on seedling phytotoxicity for MR253 rice variety.
***Vertical bars on line represent error bars with percentage (±)**

3.8 Plant Nutrient Concentration

The results showed that Cu and Fe concentration were toxic to the rice seedlings while the concentration of Mn and Zn were within limit for the rice seedlings. Furthermore, the result revealed that N concentration was deficient while P, K and Mg were sufficient for the rice seedlings but Ca was found to be at a critical level in the rice seedlings (Table 5). Nutrients contents in the rice seedling samples analyzed were compared with the critical nutrients range at tillering required by rice as reported by Dobermann and Fairhurst (2000). The analysis shows that P, K, Mg, Mn and Zn were sufficient for the rice seedling at 14 days of planting while N and Ca were far from optimum level for rice but Cu and Fe were excess and toxic in the rice seedlings (Table 6).

TABLE 5
NUTRIENT CONCENTRATIONS IN RICE SEEDLINGS AFTER 14 DAYS OF PLANTING WITH DIFFERENT CONCENTRATIONS OF MUNICIPAL WASTEWATER

Concentration of wastewater	%					mg kg^{-1}			
	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
0	1.39	0.21	1.02	0.11	0.17	32.88	468	218.44	193.33
2.5	1.41	0.24	2.41	0.15	0.17	60.22	1096	695.78	317.33
5	1.42	0.23	2.12	0.17	0.17	52.88	987.33	662.44	291.33
10	1.48	0.25	2.59	0.17	0.18	56.44	952	643.78	289.33
25	1.55	0.24	2.39	0.17	0.17	68.44	1507.33	622.44	290
50	1.66	0.24	2.49	0.18	0.17	45.55	997.33	591.78	288
100	1.49	0.25	2.08	0.19	0.18	52.66	1026.67	498.44	256.67

TABLE 6
OPTIMUM RANGES AND CRITICAL LEVELS FOR OCCURRENCE OF MINERAL DEFICIENCIES OR TOXICITY IN RICE TISSUE

Elements	Growth stage	Plant part	Optimum range	Critical level for deficiency	Critical level for excess or toxicity
N	Tillering -PI	Y Leaf	2.9-4.2%	<2.5%	>4.5%
	Flowering	Flag Leaf	2.2-2.5%	<2.0%	
	Maturity	Straw	0.6-0.8%		
P	Tillering -PI	Y Leaf	0.20-0.40%	<0.10%	>0.50%
	Flowering	Flag Leaf	0.20-0.30%	<0.18%	
	Maturity	Straw	0.10-0.15%	<0.06%	
K	Tillering -PI	Y Leaf	1.8-2.6%	<1.5%	>3.0%
	Flowering	Flag Leaf	1.4-2.0%	<1.2%	
	Maturity	Straw	1.5-2.0%	<1.2%	
Ca	Tillering	Y Leaf	0.2-0.6%	<0.15%	>0.7%
	Tillering-PI	Shoot	0.3-0.6%	<0.15%	
	Maturity	Straw	0.3-0.5%	<0.15%	
Mg	Tillering -PI	Y Leaf	0.15-0.30%	<0.12%	>0.50%
	Tillering-PI	Shoot	0.15-0.30%	<0.13%	
	Maturity	Straw	0.20-0.30%	<0.10%	
Cu	Tillering -PI	Y Leaf	7-15 mg kg^{-1}	<5 mg kg^{-1}	>25 mg kg^{-1}
	Maturity	Straw		<6 mg kg^{-1}	>30 mg kg^{-1}
Fe	Tillering	Y Leaf	75-150 mg kg^{-1}	<70 mg kg^{-1}	>300 mg kg^{-1}
	Tillering	Shoot	60-100 mg kg^{-1}	<50 mg kg^{-1}	
Mn	Tillering	Y Leaf	40-700 mg kg^{-1}	<40 mg kg^{-1}	>800 mg kg^{-1}
	Tillering	Shoot	50-150 mg kg^{-1}	<20 mg kg^{-1}	
Zn	Tillering -PI	Y Leaf	25-50 mg kg^{-1}	<20 mg kg^{-1}	>500 mg kg^{-1}
	Tillering -P	Shoot	25-50 mg kg^{-1}	<10 mg kg^{-1}	>500 mg kg^{-1}

Source: Dobermann and Fairhurst (2000)
**** mg kg^{-1} = ppm***

3.9 Plant Nutrient uptake in Seedlings

The interaction among variety, different levels of concentrations and wastewaters was significantly ($p<0.05$) different for N, P, K, Ca and Mg nutrients for MR253 rice seedlings. The concentration of N, P and K nutrients in seedlings increased when a certain concentration of treated wastewater <25% was used for imbibition. As the concentration of treated wastewater increased beyond a critical point >50%, the concentration uptakes of N, P and K decreased. The concentration of N, P and K nutrients in seedlings increased when <25% concentration of untreated wastewater was used for imbibition for MR253, thereafter, as the concentration of the untreated wastewater increased beyond a critical point >50%, the concentration of N, P and K decreased for MR253 (Table 7). The concentration of Ca and Mg nutrients in seedlings increased when a certain concentration of treated wastewater <10% and <25% respectively for MR253 was used for imbibition. As the concentration of treated wastewater increased beyond a critical point >25% concentration for Ca and >50% concentration for Mg, decreased was observed. The concentration of Ca and Mg nutrients in seedlings increased when 10% and 25% concentration of untreated wastewater was used for imbibition respectively and thereafter, as the concentration of the untreated wastewater increased beyond a critical point >25% and >50%, the concentration of Ca and Mg decreased respectively (Table 7).

TABLE 7
MEAN COMPARISON OF NUTRIENTS UPTAKE AS AFFECTED BY DIFFERENT CONCENTRATIONS, MUNICIPAL WASTEWATERS AND VARIETIES FOR N, P, K, CA AND MG FOR MR253 RICE SEEDLINGS (mg/g)

		N		P		K	
Variety	**Concentrations of wastewater**	**TWW**	**UTWW**	**TWW**	**UTWW**	**TWW**	**UTWW**
MR253	0	0.28d	0.32d	0.3b	0.34b	0.35d	0.35d
	2.5	3.90b	4.60b	0.5a	0.5a	2.90b	3.90b
	5	4.20b	5.70b	0.5a	0.6a	3.60a	4.60a
	10	5.20b	6.10a	0.5a	0.7a	4.70a	5.20a
	25	6.80a	6.50a	0.9a	0.8a	5.80a	5.80a
	50	3.70c	2.90c	0.2b	0.3b	2.01c	2.30c
	100	2.70c	2.50c	0.2b	0.2b	2.50c	2.01c
		Ca		**Mg**			
Variety	**Concentrations of wastewater**	**TWW**	**UTWW**	**TWW**	**UTWW**		
MR253	0	0.02d	0.02d	0.03d	0.02d		
	2.5	0.20b	0.50a	0.30b	0.40a		
	5	0.20b	0.60a	0.30b	0.40a		
	10	0.40a	0.60a	0.50a	0.60a		
	25	0.10c	0.50a	0.70a	0.70a		
	50	0.10c	0.20b	0.10c	0.20b		
	100	0.10c	0.10c	0.10c	0.10c		

**** Means within columns with common letters are not significantly (P>0.05) different***
****TWW= Treated wastewater; UTWW= Untreated wastewater.***

The interaction among variety, different levels of concentrations and wastewaters was significantly ($p<0.05$) different for Mn and Zn for MR253 rice seed. The concentration of Mn and Zn nutrients in seedlings increased when the seeds were imbibed in a certain concentration of treated wastewater (<25%). As the concentration of treated wastewater increased beyond a critical point (>50%), the concentration of Mn and Zn decreased. The concentration of Mn and Zn nutrients in seedlings increased when (<25%) concentration of untreated wastewater was used for irrigation and thereafter, as the concentration of the untreated wastewater increased beyond a critical point (>50%), the concentration of Mn and Zn decreased (Table 8).

The interaction among variety, different levels of concentrations and wastewaters was significantly ($p<0.05$) different for Cu and Fe for MR253 rice seed. The concentration of Cu and Fe nutrients in seedlings increased when the seeds were imbibed in a certain concentration of treated wastewater (<25%). As the concentration of treated wastewater increased beyond a critical point (>50%), the concentration of Mn and Zn decreased. The concentration of Cu and Fe nutrients in seedlings increased when (<25%) concentration of untreated wastewater was used for irrigation and thereafter, as the concentration of the untreated wastewater increased beyond a critical point (>50%), the concentration of Cu and Fe decreased (Table 8).

TABLE 8
MEAN COMPARISON OF NUTRIENTS UPTAKE AS AFFECTED BY DIFFERENT CONCENTRATIONS, WASTEWATERS AND VARIETIES FOR Mn, Zn, Cu AND Fe FOR MR253 RICE SEEDLINGS (mg/g)

		Mn		**Zn**	
Variety	**Concentrations of wastewater**	**TWW**	**UTWW**	**TWW**	**UTWW**
MR253	0	0.013c	0.040b	0.021b	0.023b
	2.5	0.120b	0.140a	0.063a	0.074a
	5	0.130b	0.140a	0.066a	0.075a
	10	0.220a	0.160a	0.069a	0.084a
	25	0.091c	0.180a	0.091a	0.086a
	50	0.046c	0.084b	0.037b	0.032b
	100	0.041c	0.053b	0.028b	0.028b
		Cu		**Fe**	
	Concentrations of wastewater	**TWW**	**UTWW**	**TWW**	**UTWW**
Variety	0	0.018c	0.010c	0.020c	0.050b
	2.5	0.066a	0.140a	0.310a	0.200a
	5	0.076a	0.120a	0.320a	0.220a
	10	0.077a	0.130a	0.370a	0.350a
	25	0.096a	0.170a	0.390a	0.450a
	50	0.051b	0.075b	0.120b	0.150a
	100	0.032c	0.037b	0.049c	0.064b

**** Means within columns with common letters are not significantly (P>0.05) different***
****TWW= Treated wastewater; UTWW= Untreated wastewater.***

These results above are consistent with the findings of Mojiri and Aziz (2011) who reported that irrigation with wastewater increased Fe, Mn and Zn in roots and leaves of *Lepidium sativum.* Arora *et al.* (2008) also observed similar results that concentrations of macro and micronutrients were higher in wastewater irrigated vegetables than in fresh water irrigated plants. The nutrients in the municipal wastewaters at the lower concentration were within the permissible limits for plant development and below those that are toxic to plant growth (Varadarajan, 1992). Increase in the concentration of different minerals in the rice seedlings subjected to different municipal wastewater irrigation was different between untreated and treated wastewater. Untreated municipal wastewater was found to have more mineral nutrients content than treated wastewater. The rice variety had more nutrient uptake when imbibed with untreated municipal wastewater compared to when imbibed with treated municipal wastewater. This might be due to the more nutrient in the untreated municipal wastewater application which might be influencing the physiological process that leads to increase in growth as compared to treated wastewater (Singh and Bhati, 2003). Furthermore, higher concentration of untreated wastewater which inhibit nutrients uptake might be due to cells that have contact with concentrated wastewater which may have high level of cell abnormalities thereby lower nutrient uptake under concentrated wastewater (Abu and Ezeugwu, 2008). Khan and Sheikh (1976) clarified in a way that significant reduction of nutrients uptake under high concentrated effluents might be due to decrease in water uptake at higher level of salinity in view of toxicity of high osmotic pressure due to high soluble salts. Similar phenomenon may have happened in this study. Crop scientists have reported that use of treated and untreated wastewater increased yield parameters of field crops to a certain concentration of wastewater and suggested that treated wastewater can be used for producing better quality crops with higher yields (El-Nahhal et al., 2013). In the present study, irrigation with both treated and untreated wastewater at lower concentration (<25%) increased nutrients uptake which helped in rice seedling growth and development.

3.10 Chlorophyll Content in Seedlings

The interaction among variety, different levels of concentrations and wastewaters was significantly (p<0.05) different for chlorophyll a and b content in the leaves of the seedlings derived from MR253 rice seed. Chlorophyll a and b contents increased at lower wastewater concentrations in leaves for MR253 rice seeds, while decrease in chlorophyll a and b contents was observed at higher wastewater for both treated and untreated municipal wastewater. Lower wastewater concentration (<25%) had promoting effect on chlorophyll a and b contents while higher wastewater concentration (50%-100%) had deleterious effect on chlorophyll a and b content for MR253 rice seedlings (Table 9).

Similar results have been reported by Garg and Kaushik (2008) in sorghum cultivars treated with textile mill wastewater. The present study was also in conformity with Pathrol and Bafna (2013) who reported decreased in chlorophyll content in *Trigonella foenumgraecum* with decrease in the dilutions of the sewage water. These findings were also similar to the findings of Khan *et al*. (2011) who suggested that higher concentrations of wastewater are inhibitory to the synthesis of chlorophyll molecules particularly chlorophyll *a*. In wheat, Liu et *al.* (2002) observed a decline in chlorophyll level when seedlings were irrigated with sewage water.

Chlorophyll a and b contents in the leaves of the seedlings decreased in response to high concentration of wastewater. Reduction in chlorophyll content induced by wastewaters could be associated with higher concentration of heavy metals (Gadallah, 1995). Some of the possible reasons for any decreases may be due to: (a) Formation of enzymes such as chlorophyllase which is responsible for chlorophyll degradation (Majumder et al., 1991: Rodriquez et al., 1987: Sabater and Rodriquez 1978). (b) Retardation of chlorophyll synthesis under the effect of heavy metals present in wastewaters or due to changes in the endogenous cytokinins in leaves (Cizkova, 1990) which were reported to be responsible for stimulation of chlorophyll synthesis (Banerji and Laloraya, 1967). (c) Enhancement of chlorophyll loss due to increases in the endogenous ABA in the leaves (Cizkova, 1990) which were reported to accelerate the chlorophyll destruction (Mittelheuser and Van Steveninck, 1971) and inhibit plastid differentiation and chlorophyll synthesis (Le Page-Degivery et al., 1987). Similar phenomenon may have happened in this study. Rice seedlings exposed to higher concentrations of wastewater (>50%) had inhibitory effect on the chlorophyll content in the leaves of the rice seedlings which can be as a result of the heavy metals in the wastewater at higher concentration which are toxic to the leaves of the seedlings. However, seedlings imbibed in lower concentration of wastewater (<25%) had stimulated effect on the chlorophyll content in the leaves of the rice seedlings. In the lower concentrations of wastewater there might be no toxic effect in the leaves of the rice seedlings rather it has some beneficial effect.

TABLE 9
MEAN COMPARISON OF CHLOROPHYLL A AND CHLOROPHYLL b (µg/ml) CONTENT AS AFFECTED BY DIFFERENT CONCENTRATIONS, MUNICIPAL WASTEWATERS AND VARIETY FOR MR253 RICE SEEDLINGS

		Chl a		Chl b	
Variety	**Concentrations of wastewater**	**TWW**	**UTWW**	**TWW**	**UTWW**
MR253	0	20.34b	20.06b	12.08b	20.40b
	2.5	26.01a	27.97a	11.83b	21.43b
	5	26.06a	29.29a	13.46b	23.33a
	10	27.62a	29.40a	15.46b	23.59a
	25	29.01a	30.75a	23.26a	32.15a
	50	21.28b	20.46b	12.57b	17.13c
	100	14.54c	12.37c	7.738c	17.87c

***** *Means within columns with common letters are not significantly (P>0.05) different*
TWW= Treated wastewater
UTWW= Untreated wastewater
Chl a = Chlorophyll a
Chl b = Chlorophyll b

3.11 Correlation Analysis

A negative and highly significant correlation was indicated for all the nutrients elements when correlated with quantity of municipal wastewater used for rice seeds imbibition (Table 10). Negative correlation indicated that when one variable is increasing the other variable has tendency to decrease. Therefore, the increase in the concentration of the municipal wastewater (>50%) saw a decrease in the nutrients elements in the rice seedlings while decrease in the concentration of the municipal wastewater (<25%) saw increase in the nutrients elements in the rice seedlings. Similar finding was also observed for rice at maximum tillering stage which showed negative correlation between P and N (Islam et al., 2008).

TABLE 10
THE VALUES OF CORRELATION BETWEEN NUTRIENTS OF RICE SEEDLINGS AS AFFECTED BY MUNICIPAL WASTEWATER

	Con	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Con	1									
N	-0.709**	1								
P	-0.737**	-0.878**	1							
K	-0.544**	-0.717**	-0.685**	1						
Ca	-0.562**	-0.736**	-0.850**	-0.596**	1					
Mg	-0.740**	-0.883**	-0.944**	-0.693**	-0.861**	1				
Cu	-0.723**	-0.848**	-0.864**	-0.614**	-0.747**	-0.846**	1			
Fe	-0.411**	-0.343*	-0.441**	-0.183**	-0.341*	-0.454**	-0.418**	1		
Mn	-0.716**	-0.845**	-0.955**	-0.665**	-0.900**	-0.950**	-0.834**	-0.470**	1	
Zn	-0.743**	-0.857**	-0.885**	-0.692**	-0.777**	-0.953**	-0.829**	-0.412**	-0.884**	1

Level of significance ** = p < 0.001, * = p < 0.01, NS = Not significant
**** Con=concentration of wastewater***

IV. CONCLUSION

The use of wastewater in plant nourishment would be beneficial water resources for irrigation due to its nutrient contents. Municipal wastewater contains essential nutrients for plant growth and development. The promotion of seeds and seedlings quality parameters at lower concentrations of the wastewater is due to the presence of optimum levels of plant nutrients in the wastewater. Seedlings imbibed with lower concentration of untreated municipal wastewater showed better seedling performance compare to treated municipal wastewater although untreated wastewater contains some hazardous toxic elements. Thus, municipal wastewaters can be used for irrigation purposes in agricultural practices after proper dilutions. It is also suggested that, treatment of municipal wastewaters is necessary to minimize the pollution effects before irrigating the crops.

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