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Startup of Climate-Smart Integrated Pest Management Against Corn Earworm *Helicoverpa zea* (Boddie) in Maize (*Zea mays L*.) for Altering Insect Risk

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Abstract:
Climate and environment changes can cause spreading of harmful insects into new areas and change their seasonal phenology, resulting in faster development and increased food consumption in ecosystem. Basically, corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), is a well-known established global pest of corn (Zea mays L.), and causes leaf injury by feeding in vegetative stage giving ragged appearance, and preferentially forages on silk tissue, ear tip and kernels of plant. An elevated CO2 can increase levels of simple sugars in leaves and lower their nitrogen content resulting an increase in damage caused by insect to consume more leaves for meet metabolic requirements. Higher temperature from global warming, lowers effectiveness of some pesticides and a greater number of pests may survive during winter season leading to spread of insect in both hemispheres. The dislodgement of earworm eggs is resulted from plants by the action of rain and wind, and these conditions influence on caterpillars that pupate into soil. Climate-smart pest management options are grow corn early to escape peak egg laying period, develop corn hybrids with silk comprising antibiotic chemical against larvae and having husks tightly fitting around ear, implement pest scouting, use economic threshold level, adopt transgenic corn hybrids, initiate Bacillus thuringiensis Berliner and nucleopolyhedrovirus sprays to target early instars of pest's population, predators and parasitoids applications, and insecticide usage as last resort. As climate change exacerbates pest problem, future management strategies include monitoring climate and pest populations, modified integrated pest management strategies, and use of modelling prediction tools.

Keywords: Helicoverpa zea, Global warming, Climate change, Crop-pest interactions, Transgenic crop, Integrated Pest Management

INTRODUCTION

Around the world, maize (**corn**) *Zea mays* L., in Family Poaceae is the tertiary utmost imperative cereal crop afterward to rice and wheat, grown for human and animal nourishment, and also used as biofuel. Maize and maize sauce are used in general diet stuffs, for instance, soft drinks and cereals, along with being used as forage for cattle. World's rising population and ethanol manufacture are growing desire for corn. Extension of pest's choices might have considerable influences by increased costs and reduced produces for pest management and seed production (Shah et al., 2015).

More than 250 insect's species have been observed, which are accompanying with maize in storage and field situations. Among these, corn earworm *Helicoverpa zea* (Boddie), is the utmost imperative limitation to corn creation. Its larva is a polyphagous and key agricultural pest, and immature attack more than 182 species of plants, including chickpea, cotton, peas, cowpea, pigeonpea, sorghum, sunflower, groundnut, tobacco, tomato, maize, field beans, and a wide range of fruits, vegetables and even tree species. This pest looks to be an important hazard to harvest harm and a key task for pest managing in corn, which is an essential diet crop (Bibi et al., 2018).

Additional vegetables, this pest will devour comprise cabbage, beans and soybeans. This is stated to as tomato fruit worm while originates on tomato, and cotton bollworm whenever originate on that crop. Characteristically, the 'worm' in sweet corn is known as corn earworm that chooses corn, nevertheless, once corn plants are not as attractive late in the season, it might harm to snap beans and tomatoes through consumption of the pods or fruits. There are several non-crop plants on which the earworm may grow initially in the season earlier to gardens and crops are planted. Cultivated hosts comprise field corn, sweet corn, rice, snap beans, green beans, eggplant, peppers, lettuce, sweet potato, strawberry, grapes and several others. Corn earworm is likewise an important pest in cannabis or hemp production, and it is not infrequent to observe larvae devouring leaves and buds (Capinera, 2001; Sarwar, 2015).

This pest species is vigorous during the whole year in subtropical and tropical environments, nevertheless progressively becomes extra limited to the hotter months by cumulative latitude. It is dispersive highly, and spreads routinely from southerly united states to northerly states and Canada. Accordingly, these zones have hibernating, both hibernating and migrant, or migrant densities, dependent on weather and location. Change in climate is creating easier things for a damaging pest, which effects maize and further crops, and corn earworm is the costliest insect and thrives in warmer weather (Fitt, 1989).

Population of earworm might boost and develop to a fresh region as worldwide climate change carries milder winters and warmer summers. Climate model repetitions suggest that wintertime will be slighter further frequently far ahead in the future, whereas hotter rising seasons will be warmer and longer more frequently than they are nowadays. Basically, in projected future climates, the number of days enough warm for the pest to breed and the number of days enough cold to die the pest. Favorable temperature and precipitation are helpful to farmers generating a good corn. However, changes to climate of the state including rising levels of carbon dioxide (CO_2), changes in precipitation amounts and patterns, and increasing temperatures in the air will upshot in numerous straight and incidental effects to the corn livelihood (DeLucia et al., 2008).

This article describes and focuses on results from studies on how expected changes in climate of a state will disturb the population of corn earworm pest and its pressure for agricultural production of maize statewide.

Life Cycle of Corn Earworm

Corn earworm is too recognized as cotton bollworm, sorghum headworm, tomato fruitworm and vetchworm. Adults moth in wingspan measure 32- 45 mm, forewings are usually yellowish brown, centrally hold a minute dark spot and distally a wide dark diagonal band, nevertheless wing border is not dark. Hind wings are basally creamy white and distally blackish, and centrally bear a minute dark spot. Adults are stated to live for 5- 15 days, however, under optimum situations might survive for above 30 days (*Mitter* et al., *1993*). Principally, the moths are nightly, generally hide in shrubbery throughout daylight hours, however, occasionally seen eating on nectar and go on vigorous during the dark period. Nearly, 3 days afterward to emergence, oviposition starts and continues till death. For oviposition, fresh-silking maize is attractive highly, however, ears with dry silk can even have eggs (Archer et al., 1994).

Eggs are pale green, usually laid singly on corn silk and leaf hairs, and with time become yellowish and then gray prior to hatch. Their outline differs from a little dome shaped to a firmed sphere, and measure around 0.5 mm in height and 0.5- 0.6 mm in diameter. Fecundity per female, varies 500- 3000 eggs and each female might give up to 35 eggs per day that hatch in around 3-4 days (Terry et al., 1987)]. Fresh caterpillars are not cannibalistic, initially quite a lot of larvae might forage together and as larvae develop, they turn out to be very hostile. But older larvae become aggressive, killing and cannibalizing other larvae, ensuing 1 or 2 larvae at each feeding spot, and habitually one mature larva in each ear of corn is found only (Braswell et al., 2019).

Customarily, earworm comprises 6 instars and lasts for12- 15 days in the hotter portion of the growing period, and bears saddle on 4^{th} segment of body. Mean widths of head capsule are 0.29 and 3.10 mm for 1 and 6 instars, while larvae lengths 1.5 and 24.8 mm, respectively. Development times average 3.7 and 2.9 days for instars 1 and 6, correspondingly. Once larva is matured, in the side of ear it bores a small earhole to leave ear and drops on soil, where pupation takes place. Fully grown, 6^{th} instar larvae leave the feeding site of host plant, make a pupal cavity 5- 10 cm beneath to ground and enter to pupal stage, which lasts for 10 to 15 days in summer. The pupae are reddish brown in hue, and measure 5.5 mm in width and 17- 22 mm in length. Adult moths appear from ground, consume liquids and nectar as food, mate and disperse to lay eggs (Herbert et al., 2003).

Overall, larva varies in color, body is green, brown, pink or occasionally yellow otherwise generally black, head has a propensity to be light brown or orange by a white net-like outline and thoracic plates are black. Usually, larva laterally holds a wide dark strip over the spiracles and a white to light yellow strip under the spiracles. A couple of thin dusky strips occurs often along the middle of the back and body bears numerous black thorn-like microspines (Figure 1). The occurrence of light-colored head and spines help to differentiate corn earworm from European corn borer *Ostrinia nubilalis* (Hubner) and fall armyworm *Spodoptera frugiperda* (Smith). Both these later commonly corn infesting species, have dark heads and lack the spines. A closely related species, tobacco budworm *Heliothis virescens* (Fabricius), has the late instar larvae, which bear microspines also. Larvae of tobacco budworm have spines on tubercles of first, second and eighth abdominal segments that are around half the height of tubercles, however in corn earworm spines are equal to one-fourth the height of tubercle (Storer et al., 2001).

Figure 1: Different color patterns of corn earworm larvae

Crops Attacked by Corn Earworm

Adults of corn earworm assemble fluid or other plant exudations from a huge number of flowering trees, plants and bush species. Along with corn, its utmost favorite vegetable hosts are asparagus, artichoke, collard, cantaloupe, lima bean, cucumber, okra, melon, pumpkin, potato, squash, spinach, broccoli, watermelon and cantaloupe. Further crops injured comprise clover, alfalfa, millet, oat, flax, sugarcane, wheat and vetch. Ornamental plants and fruits might be invaded, together with ripening peaches, plum, pear, avocado, raspberry, geranium, carnation, rose, nasturtium, snapdragon, gladiolus and zinnia (Sarwar, 2014a; 2014b).

Weeds, like crown vetch, common mallow, hemp, horse nettle, fall panicum, lambs' quarters, lupine, pigweed, prickly sida, morning glory, ragweed, purslane, Spanish needles, velvetleaf and toadflax, have been described to assist as larval hosts, along with winter vetch and crimson clover that might be equally weeds or crops, particularly cranesbill (wild geranium) species are significant weed hosts, and mostly vital wild hosts are deergrass and toadflax (Stadelbacher, 1981).

Nature of Damage by Corn Earworm

Afterward to hatching, larvae walk round the plant till an appropriate nourishing site is encountered, generally the reproductive bodies of the host plant. At what time earworms are existing, sweet corn entirely by visible new silk is vulnerable to injury. Corn during tassel phase needs not to be protected from earworms, however, crop might be invaded at that time through other pests. Anywhere, successive plantings are situated together adjacently, field having the freshest silk will possibly take the majority of egg laying, however, other fields are *not* invulnerable. Earworm is generally hard to manage if it internally feeds on reproductive bodies of plant (Terry et al., 1989; Rachel et al., 2017).

Normally, earworms only forage on kernels of sweet corn, commencing through eating at tip of ear and as they grow move downside to ear. Nearly, eating is continuously restricted to the topmost third portion of the ear. Fecal material as big soaking pills is originated in silk duct and at the tip of ear. As per fall armyworms and European corn borers occasionally do, earworms do not bore into the cob. Although earworms harm to a minor proportion of the kernels only, their droppings and presence to most of consumers are very distasteful. Wherever, control measure in the field has been fewer than perfect option, farmers are required to checkered ears at harvest and discard the injured ones. Sometimes, earworm-infested ears can be still sold if their tips are detached, though this exercise meaningfully decreases the shelf life of corn. Sweet corn intended for processing plant might be capable to withstand some damage by earworm at ear tips, because tips are not used in the finished produce (Braswell et al., 2019).

The earworm might be existing all over the season, however is abundant mostly throughout August and September. Larvae feed on leaves, whorl, tassels and within ears, nevertheless, ears are the favorite spots for attack of corn earworm. Ear injury is categorized by a widespread filth at the tip of ear. Early instar larvae forage on corn silks by cutting these off. Just afterward, larvae forage their way inside to ear, where they persist by eating in tip zone till, they exit for pupation in earth. When disturbed, they will either fight or curl into a C-shape style (Zalucki et al., 2002).

On corn, corn earworm generally feeds on every part of host, initially, younger larvae have a tendency to forage on silks and hinder in pollination, however they habitually gain entree into kernels eventually. They might forage at the tip only, or damage might spread to half the dimension of ear prior to development of larva is completed. Such type of eating likewise increases growth of plant pathogenic molds. If the ears yet have not formed silk, larvae might tunnel into the ear directly. Usually, they continue their eating within single corn ear, however, occasionally abandon the eating spot and look for another. Larvae likewise can harm to whorl-stage corn through eating on the younger, emerging leaf tissue. Nevertheless, persistence is well on more progressive development plant stages. On tomato, larvae might forage on greenery and tunnel into stalk, however, maximum of eating happens on tomato fruit. Commonly, larvae start to tunnel into a fruit, forage for a brief time only and then depart to invade one more fruit. When corn is not silking, tomato is extra vulnerable to damage, but in the existence of corn, moths will oviposit preferentially on fresh silk. Further crops, for instance, pumpkin, cucumber, bean, squash and cantaloupe might be harmed in a style analogous to tomato, but are also less possible to be wounded if silking corn is adjoining (Bilbo et al., 2018).

When larvae forage on silk and move down into kernels, this injury stops pollination and presents entry for several fungi, diseases and molds into ear. As a result, yearly produce losses range from 5-7% in field and 10-15% corn for humanoid ingesting. Besides direct injury, earworms also can hasty the crop to invasions by other pests. Afterward to earworms start eating on sweet corn grains, sap beetles will be attracting to the odor of fermenting sugars. In field corn, ear molds evolving in the injured grains can originate harmfulness complications for livestock. While larvae feed in tips of ears, consuming seeds and infecting ear, even invasions injury of the kernels less than 10% amounts, is sufficient to origin stern financial fatalities of fresh marketable sweet corn owing to customer refusal, and of hybrid dent seed corn owing to high worth of produce (Olivi et al., 2019). Larvae start eating on seeds after they have gotten third instar and enter 9-15 cm into ear, through deeper diffusion taking place as grains toughen. Larvae do not feed on tough grains, however take on chews of several seeds, thus dropping value of corn for processing (Waldbauer et al., 1984).

Impacts of Climate Change on Insect Pests

Climatic change and global warming could profoundly affect insects' life histories, favor quick establishment and development, increase both the number and appetite, expand area, and create a spare generation of progenies, which could pose a serious threat leading to a boom in pest's population. Milder winter and hotter rising season temperatures can let some of the pests to enlarge their region and create a superfluous offspring. Growing hotness increases both the appetite and number of insect and these will abolish nearly 30% further maize than they do nowadays through a rise of 2°C, but new increasing damage by pest will origin as a minimum of another 4-8% to be missing (Bale, 2002). Just by 2°C increase of worldwide warming, the global four top creators of maize will drop faraway additional to pests (Figure 2).

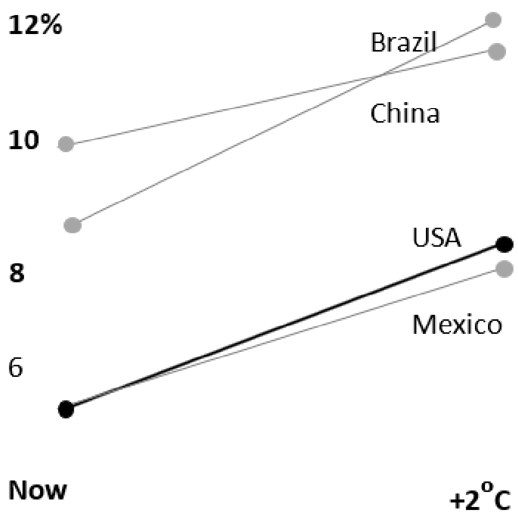

Figure 2: Percent of yield lost in Maize due to field pests - top producer country in dark color

The variation in yield is owing to expected upsurges in temperature, changes in rainfall forms and higher concentrations of surface carbon dioxide from human caused emissions of greenhouse gas, creating hard to produce more maize in the tropics. General, fatalities by 20-50% are initiate to rise for 2°C of heating above pre-industrial stages and 40%-100% for 4°C. The general photograph is that in a temperate area, if farmers are rising lots of diet, they will be going to be success toughest. Europe's breadbasket is amongst the stiffest success, by 11 countries projected to get an upsurge in pest fatalities of 75% or extra (Baker et al., 2000).

Direct environment influences on pests (life history behaviors) are well understood than indirect impact (crop-pest relations, biological control) resultant in a gap among discipline of management options and predicting changes (Furlong and Zalucki, 2017). There are some cases of transformation in topographical dispersal of few species of corn insects on account of climate change (Table 1).

Table 1: Few cases of variations in geographical spreading of insect pests because of climate change.

Insect pest	**Plant host**	**Effect on insect/ behavior comeback**	**Reference**
Old world Bollworm *Helicoverpa armigera* (**Hubner**)	Maize	Enlarged occurrence in southern Europe and epidemics, and extension of geographical expanse in northern Asia	Cannon (1998)
European corn borer *Ostrinia nubilalis* **(Hubner)**	Maize	Northward movement by per season an extra generation	Porter et al. (1991)
Corn earworm *Helicoverpa zea* (Boddie)	Maize	Range extension to high elevations, USA and northern Europe, and amplified hibernating	Diffenbaugh et al. (2008)

It has been speculated that worldwide warming will upsurge the occurrence of insect pests in several agroecosystems. This is very expected that the chief drivers of environment change (higher temperature, higher atmospheric CO_2 and reduced soil moistness) significantly can disturb density dynamics of insect pests and consequently the proportion of crop fatalities (Kocmankova et al., 2010).

INFLUENCE OF CLIMATE CHANGE ON CORN EARWORM

`There are three diverse geographic regions crossways to southern expanse, where corn earworm subsists winter; transitional zone, where it might subsist winter; and northern limits, where it is usually incapable to subsist winter for the reason of earth temperatures fall under cold. The hotter winter soil intended for insects, which live in earth are further expected to subsist (Sandstrom et al., 2007)].

The biological thresholds for an insect species joined with models of growth to determine in what way would each respond to predictable climate change circumstances, are used. For instance, pupal phase of corn earworm hibernates and cannot resist further than 5 days at temperatures under 14°F. For completion of development, it likewise needs 6 days at a temperature of around 55°F. Through including of these parameters in the climate model, an expert is capable to plan forthcoming temperature-based dispersals of pest. Corn earworm is of specific concern for the reason that it is migrant, pesticide resistant and established worldwide pest, and has particularly recognized hard to manage. The adult moth is able of being transportation lengthy distances in jet stream to invade new crops (Meola et al., 1983).

Corn earworm is well-thought-out to be amongst the maximum common farmhouse pests, destroying crops together with maize. The comfortable cold restraint can enlarge the expanse of past's taxonomic group, together with a considerable range extension in case of corn earworm, which is cold intolerant insect. Since, corn earworm is a global nuisance, it suggests that this expansion could also threaten other crops. For management of noteworthy further burden from such set of recognized insects, would necessitate an extra pest managing efforts, predictable reductions in cold limitation and upsurges in heat buildup having the potential to alter pest's management significantly. Additionally, these range extensions might have considerable financial influences by bigger insecticide and seed prices, reduced produces, and downstream effects of changes in crop harvest changeability (Dovely et al., 2016).

There are three serious foundations upsetting extent and timing of corn earworm movement; emergence period of corn earworm adult, obtainability of maturing maize as host, and obtainability of appropriate atmospheric situations for transference. Information on corn earworm movement will be useful especially in inventing and applying new control strategies in states anywhere pesticides are extensively applied to manage migrant corn earworm in seed corn, sweet corn, snap beans *Pheseolus vulgeris* L., and further crops of high value. It is important particularly to geographical range, abundance and managing of corn earworm in relation to possible climate change and usage of transgenic crops tolerant of heat and drought situations (Westbrook et al., 2010).

Response of Earworm to Increased Temperature

Physiology of insects is very delicate to variations in temperature and their metabolic degree have a tendency to nearly twofold through an upsurge of 10°C. An amplified temperature inclines to hasten insect's development, movement and consumption that can distress population dynamics through persuading geographic range, fertility, generation time, survival and density magnitude [33]. So, insect densities in tropical regions are projected to practice a reduction in growing degree on account of climate warming owing to the present temperature level that is already near to optimal for pests growth and development, despite the fact insects in temperate regions are predictable to practice an intensification in growing degree (Deutsch et al., 2018).

Hotter temperatures exponentially upsurge insects' metabolic rates and intensify propagative rates, have more insects and they will eat extra. Under a continuous vapor pressure scarcity of 8.41 mm Hg, a holding temperature of 30°C generates the utmost quick growth of corn earworm. But fertility and oviposition are more after pupae and larvae are detained at lesser temperatures (25.6 and 22.2°C) in comparison to those detained at 28.9 and 18.9°C, while a higher temperature of 32.2°C in an adjustable temperature schedule lowers egg hatching. The intermediate temperature (25°C) looks to be optimal for detection of development variances between susceptible and resistant plant matters irrespective of silk concentration. In warmer conditions, larval activity intensifies, and larval feeding and activity finish once temperatures fall under 12°C (Wiseman et al., 1989).

Response of Earworm to Higher CO_2 Concentration

Raised CO_2 and worldwide warming will disturb insects development and growth that will alter relations among insect pests and their plant hosts. Raised concentrations of atmospheric CO_2

may disturb abundance, performance and distribution of herbivore insects. Such intensifications can disturb fertility, growth rates, consumption rates and population densities of insect pests (Fuhrer, 2003). Amplified levels of CO_2 are expected to disturb plants physiology through increasing photosynthetic action, resultant in healthier development and progressive plant production. Indirectly, it would in turn disturb insects through altering both the quality and quantity of vegetation and plants. In the insect's body, nitrogen is the vital component for its growth and hence bigger concentration of CO_2 leads to upsurge plant feeding amount in some sets of pests (Bezemer et al., 1998).

Climate change is an inevitable and imminent state of affairs, driven largely by rise in temperature and CO_2. Adversative climate situations [increased temperatures (29, 31, 33 and 35± 1°C) and increased CO_2 (550 ppm)] are simulated in temperature and carbon dioxide gradient compartments for development and growth of *Helicoverpa* species, wherein relative growth rate of pest increased with elevated CO_2 and temperature (Sravan et al., 2021).

Both tolerance and resistance that are two tactics used by plants to bound biotic stress, which are disturbed through abiotic atmosphere including tropospheric levels of CO_2. The results showed that raised CO_2 reduces simultaneously the tolerance and resistance of plants to herbivore insect through lessening level of jasmonic acid, and actions of lipoxygenase polyphenol oxidase, and proteinase inhibitors in host infested with *Helicoverpa* pest (Guo et al., 2012). Thus, farmers face plague of pests as a rise in concentrations of atmospheric CO_2.

Response of Earworm to Changeable Precipitation Pattern

Variations in frequency, intensity and amount of rainfall are very vital pointers of climate change. Such kind of precipitation form has favored the incidence of floods and droughts. Species of insects, which hibernate in earth are affected directly through lapping precipitation. Significant precipitation may cause flooding and lengthy water stagnation. Such kind of occurrence impends to insect endurance and as a minimum disturbs their diapause. Besides, larvae and eggs of insects may be splashed away through flooding, heavy rains and wind (Shrestha, 2019). Insects are affected by drought because dry environments might deliver appropriate ecological surroundings for growth and development of insects, drought hassled plants attract some species of insects and are further vulnerable to insects' outbreak for the reason that of a reduction in creation of secondary metabolites having a protection role (Yihdego et al., 2019).

The limit of corn earworm's migration appears to fluctuate largely depending on temperature and precipitation during the winter. Their rate and direction of spread during the summer are expected to be affected through weather conditions. Rainfall and temperature appear to be primary limiting factors, with the probability that durations of low temperature as well as minimum temperature determine the hibernation points of corn earworm. Likewise, excessive winter rainfall has been observed to prevent hibernation, especially in the soils of low permeability. The amount of rainfall during the summer may also affect earworm populations, in that excessive amounts destroy eggs on corn silks and leaves, drown the pupae or prevent emergence of adults from soil. Conversely, the length of pupal stage may be greatly increased where drought conditions exist (Kearney et al., 2010).

While rainfall will certainly hamper migration possibilities further to west, a combination of south to southwest winds along with more scattered precipitation may lead to isolated moth flights especially into the eastern corn-growing and southern regions overnight. Fields from eastern region will be at risk of some additional isolated moth flights as southerly winds return along with potential precipitation chances leading to isolated drop zone regions. In many areas of the northern corn-growing region, there have seen corn earworm moth flights. So, growers should continue to monitor traps and scout fields especially where crop stage is susceptible to damage (Staley et al., 2007).

Moths of corn earworm are capable to scatter up to several hundred kilometers by means of periodic breezes, meaning that these may disperse fast when circumstances are upright. The hotter season soils inevitable to insects, which live in soil are likely to continue more. Globally, the 2°C (3.6°F) of heating would enhance appetite and number of insects triggering them to abolish 30% extra maize. Earworm spends wintertime underneath to ground and is not recognized to subsist in conditions beyond a 40 degrees latitude of north, however this is altering as soil warms and it disperses to newer regions (Porfirio et al., 2014).

Climate Changes Accelerate Corn Earworm's Resistance

Worldwide, climate changes have noteworthy effects on farming and likewise on farming insect pests. Farming crops and their equivalent insects are indirectly and directly influenced through environment change. Direct influences are on pests' development, reproduction, dispersal and survival, while the climate change indirectly affects relations among pests, their atmosphere and other insect species, for instance, vectors, natural enemies, mutualists and competitors (Tilman et al., 2011). During the past some years, because usage of Bt corn has increased, corn earworm progressively established more resistance to insecticide properties of heritably altered corn that is engineered to hold a protein from *Bacillus thuringiensis* (Berliner) bacterium, which is lethal to several insects, however inoffensive to people, and study arguments to climate change as a reason of hastening the resistance development in pest. For instance, surges in temperature have caused in an amplified overwintering survive and range expansion of corn earworm (*H. zea*) and cotton bollworm (*Helicoverpa armigera* Hubner). Accordingly, this looks to be a noteworthy menace to foremost task for pest managing and harvest harm in essential food corn crop (Fand et al., 2012).

Specifically, high Bt corn acreage and exceeding normal temperature correlate by upsurges in injury to corn ear, grain ingesting of Bt corn, and share of late instars of *H. zea* noted. The spreading hazard of Bt-resistant corn earworm and its damages associated to several crops is higher for the reason that corn earworm overwintering range might further expand owing to climate change, and evolutionary selection pressure for resistance expansion applied through extensive Bt acreage. High temperature, after combined with higher use of Bt corn, might destroy Bt proteins in crops; hasten insect development permitting *H. zea* to progress each season by more generations; letting *H. zea* to subsist more overwintering; and leading to northward extension of resilient insects (Venugopal and Dively, 2017). The corn earworm harm to corn ears, grain portion consumption, average instars and number of later instars in Bt varieties are increased with temperature irregularity and Bt adoption, through interactive or additive influences (Gore et al., 2002).

MANAGEMENT OF CORN EARWORM IN CLIMATIC CHANGES

For agricultural producers, creation logic of what is happening with corn earworm is actually imperative. There are many possibilities for management of corn earworm and controlling options vary dependent on which crop is being injured. On sweet corn and field corn, on silks eggs are laid, freshly hatched larvae forage downward to silk channel and then on tip of ear. In this circumstance, there is slight chance to usage insecticides for the reason that larvae are in protected places. When insecticides are to be applied, these must be used at the period of egg laying, generally by repetitive applications from period of silking till afterward to brown silk phase is gotten (Gregory et al., 2009; Sarwar, 2013a).

Climate-smart pest management reflects climate change influences on entire features of wider habitats and farming system, along with in what way these interact with existing and new risks of insect. The furthermost commonly earworm's managing strategies are monitoring of climate and insect pest population, modified integrated pest management practices, and usage of modeling predictions devices (Raza et al., 2014).

Monitoring of Corn Earworm

A global system for sharing of data on insects, hostile alien species and weather information is need, and be shared between local, regional, national and international levels to improve cooperation between countries. Generally, on corn, eggs and larvae are often not appraised for the reason that eggs are actually hard to notice and soon after hatching larvae tunnel downward into silks resulting out of the insecticides reach. Moth of corn earworm can be checked with installation of pheromone and light traps. Both sexualities are arrested in light traps, while males are only lured to sex pheromone. Both types of traps bring an estimation of at what time moths emerge or invade the crop and give their relative concentrations, however, pheromone traps are easy to usage since these are selective. Usually, the pheromone is usage in combination with an upturned cone-type trap. Normally, the occurrence of 5- 10 moths for every night is adequate to stimulate pest control practice. First generation populations may or may not reach economic levels, however, once the second-generation hits, populations will likely be high for the rest of the season (Studebaker et al., 1991).

Sampling visually for larvae and eggs is the core procedure used for routinely sampler in corn. Sweep netting is an easy and quick way for sampling maximum of other crops. Monitor crops for pest's activity through taking of at least 5 sets of 10 sweeps and calculate the mean number of larvae per 10 sweeps. The usage of pheromone traps that only attract males, may offer an early caution of moths coming to a zone or their appearance from local wintertime diapause. These must be established at the beginning of spring and earworms are generally monitored by using of a *Heliothis* trap. A pheromone lure (corn earworm Luretae) that reproduces the female earworm's sexual attractant, is sited in the trap. Male earworm moths are lured to the pheromone and trapped inside the trap. Because pheromone traps catch only males, they cannot be used to resistor an invasion (Ahmad and Sarwar, 2013).

Ideally, there should be put the trap just before the corn begins to tassel and these are set just outside of crop on southwest corner of field, because that is the track where predominant breezes blow. One trap is sufficient for each farmhouse and pheromone lure must be altered after each 2-3 weeks. Generally, an insecticide spray is applied at early silking as soon as the first corn earworm moth is captured on the farm and applications are repeated at 2- 6-day intervals based on moth pressure and corn hybrid (Sarwar, 2017a).

Modified Integrated Pest Management (IPM) Approaches

Integrated pest management (IPM) offers stable and continuous destruction of pests through encouraging their natural enemies. This long-lasting tactic is likewise the minimum lethal technique for insects controlling. Chemicals, only used as per a latter option normally are the least required. Cultural practices, host plant resistance, monitoring, natural enemies and synthetic insect killers are nowadays being used widely for pests managing. But, several of these approaches for pests controlling are sensitive to the atmosphere exceedingly. Hence, there is a necessity to raise suitable stratagems for pests managing, which might be operative under circumstances of future global warming (Sarwar, 2004). Presently existing pests managing tactics, for instance, host plant resistance, cultural control, biological control and chemical control along with use of biotechnology could be intensified in response to changing climate (Khan et al., 2020).

Cultural Control:

Trap cropping is frequently proposed for earworm; the higher grade of preference for corn in green silk period by ovipositing moths may be used for lure of moths to lesser favorite crops. Lima beans are comparatively more striking to attract moths, in comparison to tomato plant. Chickpeas as a trap crop may also be used to capture overwintering populations emerging from diapause. Nevertheless, it is hard to continue an attractant crop in an attractive phase for a prolonged period. In an area where population grows initially on weeds host and then scatter to crops, handling of weeds done by cutting, weedicide or applications of insecticide against earworm can critically amend harm on adjoining crops (Sarwar, 2011; 2019).

In certain expanses, sometimes it is probable to grow or reap crop enough early to escape damage. All over the expanse of this insect, its inhabitant densities are at peak and utmost destructive late in the growing period.

Ploughing, particularly in the autumn, significantly can decrease hibernating activity of pupae in some places. Tilling of grounds would kill numerous of the hibernating pupae. But this insect is extremely moveable and migrant reinvasion can be predictable from distant sources. Slashing and cultivating can lessen its larval and pupal survivals in field (Sarwar, 2017b).

Host Plant Resistance:

Resistance of corn to earworm is derivative from physical features such as ear length and husk tightness that hinder entree to ear kernels by larvae, or chemical features like maysin that prevent growth of larvae. Hereditarily engineered assortments of few crops are available now, which comprise *B. thuringiensis* toxin in plants that decreases harm by *H. zea* and damage to corn (Burkness et al., 2001; Sarwar, 2013b). There are certain varieties of sweet corn that express some resistance to damage by corn earworm. Grow mid-season assortments, which will mature among moth flights, so the ears will likely to be less injured. Productive landscapers on their corn can stop moths from laying eggs, by applicating 20 droplets of mineral oil by a medicine dropper to silks within tip of ear (3- 7 days later to silks first seem) till silks have wilted.

Preferably, host plant resistance to insects is one of the most environment friendly apparatuses of pest managing. So, it is imperative to detect and progress cultivars, which are stable in resistance expression under variable climate to target pests. There is necessity to combine host plant resistance from germplasm with the transgene expression showing stability across locations and environments (Tabashnik et al., 2013; Khalid et al., 2015).

Biological Control:

Biological control is more fruitful at that time when earworm is eating on leaf or outside of fruiting structure (Sarwar, 2016a). Amongst its commonly hunters are ladybird beetles (Coleoptera:

Coccinellidae) pink spotted lady beetle *Coleomegilla maculate* DeGeer and convergent lady beetle *Hippodamia convergens* Guerin-Meneville (Sarwar, 2016b; 2016c); big-eyed bugs *Geocoris* spp. (Hemiptera: Lygaeidae); softwinged flower beetles and *Collops* spp. (Coleoptera: Melyridae); minute pirate bug *Orius tristicolor* (White) (Hemiptera: Anthocoridae); and green lacewings *Chrysoperla* and *Chrysopa* spp. (Neuroptera: Chrysopidae) (Sarwar et al., 2011; Sarwar and Salman, 2016).

The bacterium *Bacillus thuringiensis* Berliner or *Bt* bacterium provides some suppression and sprayable formulations may be useful when a minimum lethal control way is wanted. Now, inherently engineered assortments of certain crops are available, which include *B. thuringiensis* toxin that lessens injury by earworm (Sarwar, 2015a; Sarwar, 2021).

Commercially available, entomopathogenic nematodes, deliver upright destruction of emerging larvae when they are applied to corn silk. Earth outward and subsurface applications of nematodes can also disturb earworm densities due to the reason that larvae fall to soil for pupation. Nematode *Steinernema riobravis* (Nematoda: Steinernematidae) is noted to be an important factor of mortality for pupae and prepupae. Likewise, *Heterorhabditis heliothidis* (Nematoda: Heterorhabditidae) is also observed for parasitizing of corn earworm (Sarwar and Mukhtar, 2021). Pathogenic fungus *Nomuraea rileyi* and *Helicoverpa zea* nuclear polyhedrosis virus are generally intricated in occurrences of illness in earworm (Sarwar et al., 2021a), however protozoan *Nosema heliothidis* as well as other viruses and fungi have also been detected (Sarwar et al., 2021b).

Egg parasitoids *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are raised and released for destruction of earworm in quite a lot of crops. The crop hosts seem to influence parasitism rates of earworm, by tomato being a specifically appropriate crop for releases of parasitoids (Khan et al., 2010; Sarwar, and Salman, 2015a).

Used for a slighter degree, *Telenomus* spp. (Hymenoptera: Scelionidae) are commonly parasitoids of egg. Common parasitoids of larva comprise *Campoletis* spp. (Hymenoptera: Ichneumonidae); *Microplitis croceipes* (Cresson) and *Cotesia* spp. (Hymenoptera: Braconidae); as well as *Archytas marmoratus* (Townsend) and *Eucelatoria armigera* (Coquillett) (Diptera: Tachinidae) (Sarwar, 2020a).

Crop divergence is among the greatest active procedures for enhancing the abundance and activity of usual enemies. There is necessity to create plant cultivars that are friendly to the survival of natural enemies, along with detecting cropping schemes, which may boost diversity of natural enemies for pest managing (Sarwar, 2020b).

Chemical Control:

Chemical sprays are used to defend corn, on the other hand after the larvae have passed in the ear, there is no an active controller. About to happen effectively, grower needs to cover end of ear completely, with the intention of when eggs hatch, early caterpillars will instantly contact to fatal dosage of insect killer (Sarwar, 2015c; 2016d; Sarwar and Salman, 2015b). Thus, actions might be reapplied every 3- 4 day from once silks initially look till they turn out to be brown.

Spray when all subsequent circumstances are factual; smaller caterpillars seem in sweep net, smaller caterpillars originate all over the field and new ears are existing on plants. If ears are not preserved reasonably, growers always can just detach the spoiled portions of infested ears, as the not fed portion by caterpillar is still flawlessly upright. Improved insecticides application, usage of resilient hereditarily altered crops and practices like crop rotations are helpful to resistor suffering from insects. However, it still looks that virtually under entire climate change circumstances, populations of pest will be the victors (Sarwar, 2015d; 2016e; Sarwar and Sattar, 2016).

There is essential for a bigger thoughtful upon the upshots of climate change on efficiency of artificial insecticides and their persistence in atmosphere. Hence, there is necessity to advance insecticide preparations and application timetables that would be minimum affected through climate change. Lastly, it needs to practice an integrated pest managing system by taking into attention the alteration in pest range, cropping outlines and efficiency of diverse apparatuses of pest managing for workable crop creation (Sarwar, M. 2020c).

Biotechnology Usage in Corn:

Biotechnology for corn has been used in two prime ways; Bt corn that has been altered genetically to express one or more proteins from lethal bacterium *B. thuringiensis* to manage destructive insects, like corn earworm, western bean cutworm, corn rootworm and more; and herbicide tolerant corn, altered for tolerance to some herbicides within their fields during the growing period. Both kinds of these corn qualities propose numerous profits to grower and atmosphere to decrease the necessity for spray of insecticides for control of insects as well as herbicides to avoid weeds during the growing season (Noreen et al., 2021). Nevertheless, before final sanction of seeds created by biotechnology, stock can be grown in field and might be undertaken severe approval procedures at Food and Drug Administration, Department of Agriculture as well as Environmental Protection Agency.

Climate Forecasting and Development of Model

Through monitoring of pest and climate, together with pest and climate risks forecast information, agriculturalists may defensively implement confident practices for insects' prevention to decrease incidence and surge of probable pest difficulties. A precautionary management package to counter corn earworm might commence as soon as 10% of ears are silked. Repetitive sprays at 3- 5 day breaks till 90% of silks have wilted would give a higher proportion of worm free ears throughout initial and midseason periods. Controlling of pest is further problematic during late period of crop growing. Even reducing spray breaks might only produce 90% of insect free ears (Duffield and Dillon, 2005).

Climate models in combination with ecological necessities of a specific pestiferous species like earworm might be an active device for protruding the probable series of variations on worldwide basis. Modelling the pest's menace along with comebacks of its host plants to climate change may thus surge the capability to forecast the consequence of an insect invasion (Heeb et al., 2019).
These models detect statistical relations between climate variables and present geographical dispersals of a specified species, which at that point are applied to forecast climate change in the future for suggesting climatically appropriate territories for that species. The ultimate productivity of models is frequently offered in the system of maps displaying forthcoming climatically suitable areas for a specified species, overall expanse of which at that time can be connected to existing geographical arrays for estimating upcoming hazard of their establishment and introduction (Evans et al., 2015). This is better that insect pest movements range are predicted to progress results that alleviate crop damage in extension expanses. Hence, there is an excessive necessity for forecasting and framing adaptation tactics in the form of improved IPM strategies, pest and climate monitoring, and usage of modelling apparatuses. Additionally,

formation of farming cooperatives is the only way to consolidate and facilitate integrated management of pest, and governments should act as facilitators in providing of incentives and training farmers in cooperative crop management.

CONCLUSION

Broadly, this article highpoints the tasks for pest's managing possibilities, for instance, against corn earworm, in the framework of environment changes. For the study of climate change scenario, procedures for monitoring and slowing the growth of resistance within such corn pest is critical. Pheromone trap information give initial cautioning of invasion and will as well attentive handler to hit a small population level prior to it converts severe, wherein lure could be applied best by Delta trap. Sampling of corn earworm for testing and monitoring resistance is concentrated currently in some areas, wherever together Bt corn and Bt cotton are grownup. It needs to expand monitoring of resistance in entire high corn creation zones and as well stress to integrate evolutionary procedures inflated by climate change into resistance managing plans. Significant rises in pest harm prediction, necessitate action to be taken during climate change and adaptation strategies. Everyone comprising wider society, farmers, policymakers and industries must be involved in planning and formulating of improved IPM strategies, pest and climate monitoring, and usage of modelling devices. Precisely, upshots of climate change on pests and their crop hosts have to be computed further to advance reasonable pressure mitigation approaches.

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Influence of Slope Position on Soil Carbon Stock and Selected Soil Fertility Indices in Lowland Rain-Fed Rice Field

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Abstract:
Most farmlands in Southeastern Nigeria are associated with low organic matter content and are prone to soil erosion and nutrient loses due to runoffs from undulating soil surfaces. This consequently limits the capacity of these farmlands to produce abundant food. The present study investigated the effect of slope position on soil organic carbon (SOC), carbon stock and selected fertility indices of lowland rice fields in Ebonyi State, Nigeria. This study was conducted in four lowland-rice fields/location in Ishiagu, Ebonyi State, Nigeria. In each location, three slope positions (upper slope, middle slope, and lower slope) were identified. Topsoil samples (0-15 cm) were collected in quadruplicates from each slope position for soil chemical analyses. Results showed significant differences in the soil physicochemical properties across slope positions and locations. The middle slopes had higher ($p < 0.05$) pH, cation exchange capacity (CEC), and total nitrogen than the upper and lower slopes. Carbon stock varied from 4.22-13.0 t C/ha across slope position but was insignificant across locations. Upper slopes had higher (11.4 g/kg) SOC than lower slopes (9.68 g/kg). The study locations had low SOC, CEC and soil nutrients; an indication of soil erosion impact in that area. The results of study reveal that organic amendment application is needed to build-up the SOC, which is crucial to preventing soil erosion, nutrient losses, as well as enhancing crop productivity and improving the overall soil health.

Keywords: Carbon sequestration, Farming systems, Food security, Landscapes, Soil erosion

INTRODUCTION

Ebonyi state, Nigeria, is renowned for its rice farming. Rice production in Ebonyi State depends on rain-fed conditions, which is susceptible to climate variabilities that affect yield performance. About 93% of cultivated land in sub-Saharan Africa depends on rain-fed agriculture (FAO (2002). This suggests that rain-fed agriculture plays a crucial role in food security and water availability. Kadigi et al. (2004); Wani et al. (2009); Nwite et al. (2015) opined that rain-fed agricultural lands vary on the amount and distribution of rainfall in an area. Rain-fed lowlands are typically faced with poor soil quality, drought/flood conditions, and as well as unsustainable management practices that negatively affect yields (Meertens et al., 1999; Devendra, 2016). Most farmlands in Ebonyi state are prone to soil erosion, which results to nutrient loses due to runoffs from the undulating soil surface. This has serious negative impact on rice growth and yield performance. Further, most farmers often cultivate without applying adequate agricultural inputs (such as NPK fertilizers, manures or composts) to enhance soil nutrient or at least to restore soil nutrients lost to plant nutrient uptake and soil erosion. Such land use management practices negatively affect soil organic carbon, accelerate soil degradation, erosion of steep slopes (Gabiri et al., 2018) and have negative environmental effects (Wezel et al., 2007). This is because water movements erode unprotected slope surfaces and wash away soil nutrients, which are reallocated in the watershed (Dung et al., 2008; Pansak et al., 2008; Aung et al., 2013).

Soil erosion is the single largest threat that has serious negative effect on the productivity and sustainability of inland valleys/lowland rice fields in southeastern Nigeria (Sullivan, 2004). Most rice fields in Ebonyi state are located on sloppy lands with varying degrees of elevation. Hence, sediment rich water flows into the paddy fields from upper paddy side and flow out through the lower paddy. The differences in slope position cause differential influence on soil properties and hydrological conditions (Hseu and Chen, 2000); Tsubo et al., 2006; Gabiri et al., 2018). This consequently affects crop yield and productivity due to uneven sediments distribution and spatial variability in soil fertility of downstream watershed (Gao et al., 2007; Mingzhou et al., 2007; Gabiri et al., 2018). Soil varies considerably from place to place, across landscapes, both vertically and horizontally (Wilding, 1985; Ezeaku and Eze, 2014). Bockheim (2005) opined those soils formed on the same parent material within an ecological zone are intricately linked and can exhibit considerable variations in soil properties. Mojiri et al. (2018) reported changes in soil properties and productivity along a toposequence. Slope positions vary heterogeneously in morphology (physiography), soil type, vegetation, and hydrology (Mbagwu, 1995; Teka et al., 2015). Variations in soil properties across landscapes affect crop productivity and yield due to anthropogenic and natural activities (Xiao et al., 2016), which affect soil organic matter and nutrient reserves (Ezeaku and Eze, 2014; Rallos et al., 2017; Gabiri et al., 2018). Rossiter (1994) emphasized the importance of topographic position in land evaluation in predicting land performances. However, the effect of slope position on carbon stock and soil fertility attributes under intensive rice cropping in Ebonyi state have not received much research attention. This study aimed at evaluating changes in soil carbon stock and some soil physicochemical properties of a derived savanna as affected by slope position in lowland rice fields of Ebonyi state.

MATERIALS AND METHODS

Study Location

The study was conducted in four lowland rice fields/locations (Amaeze, Ovumte, Federal College of Agriculture Ishiagu [FCAI] and Fallow, at Ishiagu) in Ebonyi atate, Southeastern Nigeria. A split-plot factorial arranged in randomized complete block design (RCBD) was used for this study. The main plots were slope positions while locations were the sub-plots. The study area, a derived savanna zone, is located between latitude 5° 55′ N and 6° 00′ N and longitude 7° 30′ E and 7° 35′ E, has a low-lying and undulating relief (Ezeh and Chukwu (2011) and a bimodal pattern of rainfall pattern. The annual rainfall ranges from 1250 mm to 1500 mm with a mean annual temperature of about 27°C to 28°C. The relative humidity is 80% during rainy season and 65% in the dry season (ODNRI, 1989). The area is characterized by rampant flooding and water logging due to poor drainage which is caused by an impervious layer, high soil bulk density and crusting (Nwite et al., 2014; FDALR, 1985). Flooding occurs at the peak of rainy season (July and October) and it covers the basins and floodplains around the middle and lower river and stream courses (Nwite et al., 2014). The major land uses include rice farming mainly during the raining season, citrus and oil palm plantations, multiple (annual) cropping of other arable crops and vegetables during the off-peak rainy reasons.

Soil Sample Collection

In each study location, three slope positions (upper slope, middle slope, and lower slope) were identified. Topsoil samples (0-15 cm) were collected in quadruplicates from each slope position for soil chemical analyses after being were air-dried, crushed and sieved with a 2 mm sieve. Undisturbed soil samples were also collected with the aid of cylindrical (5×5 cm) cores from each slope position for soil bulk density determination.

Laboratory Methods

Particle size distribution was determined by hydrometer method as described by Gee & Bauder (1986). Soil pH in H_2O and KCl was determined using McLean (1982) method. Soil organic carbon (SOC) was determined by Walkley and Black method as described by Nelson and Sommers (1996). Total nitrogen was determined by semi-micro Kjeldahl digestion method using sulphuric acid and $CuSO_4$ and Na_2SO_4 catalyst mixture (Bremner and Mulvancy, 1982). Cation exchange capacity was determined using Rhoades (1982) method. For bulk density (BD) determination, core soil samples were collected and allowed to drain freely for 24 hours, oven dried and thereafter calculated thus: Bulk density (g/cm^3) was determined as described by Blake and Hartge (1986) method.

$$\text{Bulk density} = \frac{\text{Mass of dry soil (g)}}{\text{Volumn of soil (cm}^3\text{)}}$$

Carbon stock (t C/ha) was calculated using the equation:

$$\text{Carbon stock } = \frac{\text{Carbon x soil bulk density x area x soil depth}}{100}$$

DATA ANALYSIS

Data obtained was subjected analysis of variance (ANOVA) using GenStat 3 7.2 Edition. Treatment means were separated and compared using Least Significant Difference (LSD) and all inferences were made at 5% probability level. Slope positions and locations were subjected to simple linear regressions to investigate their relationship with SOC stock.

RESULTS AND DISCUSSION

Particle Size Distribution Across Slope Positions and Locations in Lowland Rice Fields

Results of the particle size distribution show that fine sand varied from 3% to 53%; clay content ranged from 9% to 53%, while the silt content ranged from 19% to 47% (Table 1). At the middle slope, Ovumte had the highest (47%) silt content while Fallow had the highest (53%) clay content at the upper and lower slopes.

The results show that Amaeze, Ovumte, FCAI and Fallow were composed of clay loam, loam, sandy loam and sandy clay respectively at the upper slope position. However, at the middle slope position, Amaeze and Ovumte were composed of clay loam while FCAI and Fallow were of sandy loam and clay textural classes respectively. At lower slope position, Amaeze and Fallow were of clay textural class while Ovumte and FCAI had clay loam and sandy loam textural classes, respectively. Soils of the study locations were predominantly gravelly sandy loam and clay loam texture. This is due to the sandy shales, with fine, grained micaceous sandstones and mudstones that characterized the soils of this area. Study has shown that the texture of any soil type is due to its parent material (Igwe et al., 1999).

Table 1: Selected physical properties of the soils of Ishiagu lowland rain-fed rice fields

Locations	Slope positions				
	Upper Slope				
	Textural class	% Clay	% Silt	% Fine sand	% Coarse sand
Amaeze	Clay	33	33	25	9.0
Ovumte	Loam	19	45	35	1.0
FCAI	Sandy Loam	9	19	35	37
Fallow	Sandy Clay	53	43	3	1.0
	Middle Slope				
Amaeze	CL	29	33	29	9.0
Ovumte	CL	29	47	23	1.0
FCAI	Sandy Clay Loam	20	23	53	4.0
Fallow	Clay	49	33	17	1.0
	Lower Slope				
Amaeze	Clay	43	33	21	3.0
Ovumte	Clay Loam	29	37	33	1.0
FCAI	Sandy Loam	11	25	32	32
Fallow	Clay	53	29	17	1.0

FCAI = Federal College of Agriculture Ishiagu

Effect of Slope Positions and Locations on Carbon Stock

There were differences in carbon stock across slope positions and locations (Table 2). The highest (9.57 t C/ha) mean carbon stock was obtained in the upper slope positions. Across upper slope, Ovumte had the highest ($p < 0.05$) carbon stock while Fallow had the least carbon stock (Table 2). Although no significant changes in carbon stock were observed across slope positions, the significant variations in carbon stock across locations, suggests that the land use types and soil management practices employed by farmers contributed to varied carbon stock obtained. Wanshnong et al. (2013) reported that alterations in land use can have negative environmental impact such as (accelerated soil degradation due to erosion) which can result organic carbon loss in steep slopes.

Table 2: Influence of slope positions and locations on the soil carbon stock

Slope positions	LOCATIONS				Mean
	Amaeze	Fallow	FCAI	Ovumte	
Carbon stock (t C/ha)					
Upper slope	11.8	6.21	7.32	13.0	9.57
Middle slope	6.50	6.94	13.5	8.43	8.83
Lower slope	4.22	12.4	7.80	6.72	7.78
Mean	7.50	8.50	9.53	9.38	8.73
LSD (0.05) slope positions				NS	
LSD (0.05) locations				2.045	
LSD (0.05) slope positions x locations				2.952	

FCAI = Federal College of Agriculture Ishiagu; LSD = Least significant difference

Irrespective of location, soil C stock correlated positively ($R^2 = 0.9901^*$, Figure 1) with slope positions. It therefore suggests that the high carbon sequestration recorded at upper slopes than lower slopes (Table 2, Figure 1) could be attributed to the improved system of rice farming, *sawah* technology, that is adopted by smallholder farmers in Amaeze and Oveumte locations. Sawah rice farming technology refers to levelled rice field enclosed by bunds having inlets and outlets for irrigation and drainage (Nwite et al., 2016) with the aim of efficient controlled water supply and its utilization within the field using bunds and structural embankments.

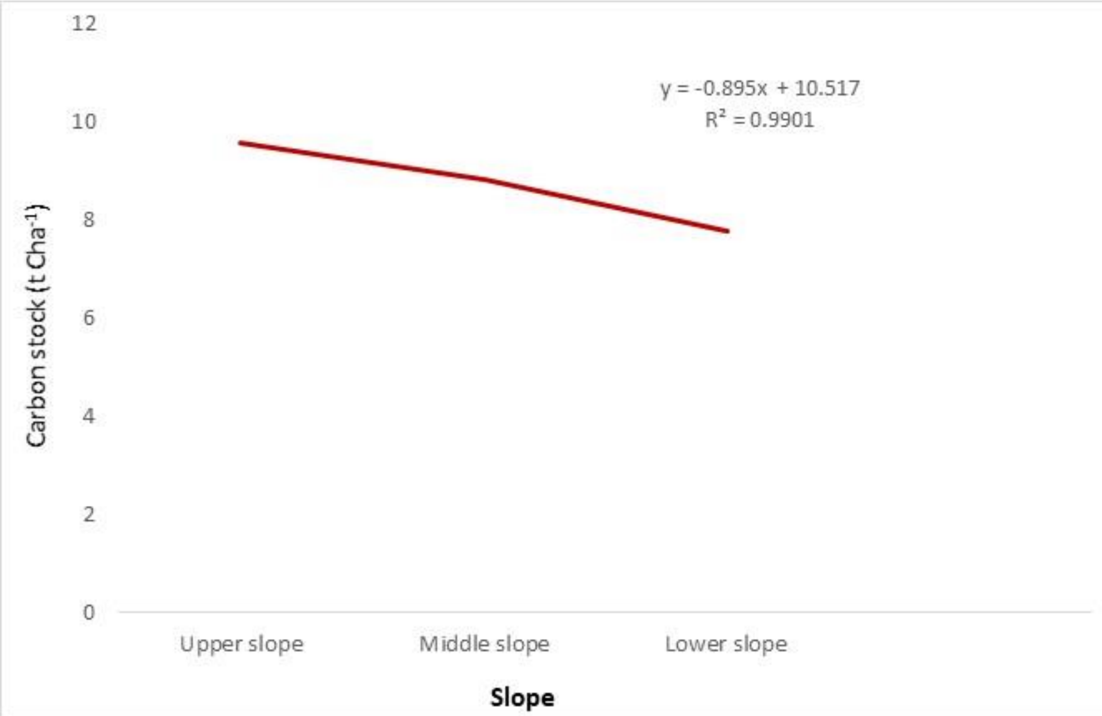

Figure 1: Relationship between slope positions and carbon stock (t C ha^{-1})

Influence of Slope Position on Soil Organic Carbon, Ph, Total Nitrogen and Cation Exchange Capacity

There are significant ($p < 0.05$) variations in the soil organic carbon (SOC) across slope positions and locations (Table 3). For Amaeze and Ovumte, upper slope position recorded higher ($p < 0.05$) SOC, while the lower slope position had the lest ($p < 0.05$) SOC. The SOC recorded for Fallow was higher ($p <0.05$) at lower slope than at upper slope position. The high SOC associated with lower slope position for Fallow can be attributed to cumulative impact of runoff, which eroded the upper slope and deposited the eroded sediments at the valley bottoms.

This is because, the runoff process may have been ongoing at the time the location was under cultivation till time the location was fallowed. Overall, FCAI gave the highest (12.4 g/kg) mean SOC while Fallow lowland gave the lowest mean SOC (9.33 g/kg). The low mean SOC obtained from Fallow relative to those of Amaeze, Ovumte and FCAI is due to annual bush burning practice by the villagers.

Slope positions and locations interaction significantly ($p <0.05$) influenced SOC (Table 3). This present result corroborates the study by Wanshnong et al. (2013) who found high SOC concentration at the top slope. According to Laurance et al. (1999); Porder et al. (2005); Nardoto et al. (2008), tropical lowlands display notable heterogeneity in nutrient cycling and nutrient constraints on ecosystem processes vary from local to regional scales within humid lowland forests (Kaspari et al., 2008; Townsend et al., 2011).

Table 3: Influence of slope positions and locations on the soil organic carbon, soil pH, total nitrogen and cation exchange capacity in lowland rain-fed rice fields

Slope positions	LOCATIONS				Mean
	Amaeze	Fallow	FCAI	Ovumte	
Soil organic carbon (g/kg)					
Upper slope	15.4	7.47	9.51	13.4	11.4
Middle slope	8.49	6.83	17.4	10.3	10.8
Lower slope	6.5	13.7	10.2	8.31	9.68
Mean	10.1	9.33	12.4	10.9	10.6
LSD (0.05) slope positions	0.594				
LSD (0.05) locations	0.644				
LSD (0.05) slope positions x locations	1.052				
Soil pH					
Upper slope	5.3	5.0	5.0	5.0	5.1
Middle slope	5.0	5.1	5.2	5.0	5.1
Lower slope	5.1	4.8	5.0	4.7	4.9
Mean	5.13	4.94	5.08	4.88	5.01
LSD (0.05) slope positions	0. 065				
LSD (0.05) locations	0.067				
LSD (0.05) slope positions x locations	0.110				
Total nitrogen (g/kg)					
Upper slope	1.31	0.79	1.06	0.92	1.02
Middle slope	1.87	1.1	1.57	0.93	1.37
Lower slope	1.19	1.07	1.24	2.1	1.4
Mean	1.46	0.99	1.29	1.32	1.26
LSD (0.05) slope positions	0.156				
LSD (0.05) locations	0.176				
LSD (0.05) slope positions x locations	0.285				
Cation exchange capacity (cmol/kg)					
Upper slope	22.2	10.7	14.6	19.5	17
Middle slope	25.7	9.2	23.5	14	18.1
Lower slope	12.5	14.8	20.1	11.3	14.7
Mean	20.2	11.6	19.4	15	16.5
LSD (0.05) slope positions	0.876				
LSD (0.05) locations	0.579				
LSD (0.05) slope positions x locations	1.093				

FCAI = Federal College of Agriculture Ishiagu; LSD = Least significant difference

Locations and slope positions interactions influenced SOC significantly. Differences in soil types (Table 1) and contrasting climatic conditions might contribute to the observed differences. Salako et al. (2006) reported no significant interaction effect between locations and slope positions on SOC. The results of the present study indicate that slope positions strongly affect C stabilization (Figure 1).

Our finding corroborates the report by Hancock et al. (2010), who found a strong and significant relationship between SOC and slope position. Variations in soil properties along toposequences has been reported (Hattar et al., 2010, Umali et al., 2006; Negasa et al., 2017). Across slope positions and locations, the soil pH ranged from from 4.7 (Ovumte, lower slope) to 5.3 (Amaeze, upper slope). Overall, upper and middle slope positions had higher mean pH than lower slope position (Table 3). Across the location, Amaeze had the highest ($p < 0.05$) mean pH value (5.13) while Ovumte had the lowest mean pH value (4.88). The variations in soil pH is linked to different land use practice and land management systems undertaken in the study areas.

The result corroborates the findings of Wilding (1985) and Ezeaku and Eze (2014), who reported that soil pH varies spatially within and across agriculture fields. Soil total nitrogen (TN) differ significantly ($p < 0.05$) across slope positions and locations (Table 3).

Except for Ovumte, middle slopes were associated with higher TN concentration than lower slope and upper slope. Overall, the upper slopes had lower ($p<0.05$) TN relative to other slope positions.

The significantly low TN associated with the upper slope can be attributed to the effect of runoff due to soil erosion which wash off soil nutrients and deposit eroded nutrients at watersheds (Aung et al., 2013; Dung et al., 2008, Pansak et al., 2008). Deposited sediments create patterns of spatial variability in soil fertility of downstream watershed (Gao et al., 2007; Mingzhou et al., 2007). Across the locations, Amaeze had highest (1.46) mean TN concentration while the fallow lowland had the lowest (0.99) TN concentration (Table 3).

The low TN in fallow is due to denitrification process that resulted from frequent bush burning. This result agrees with the submission of Ayeni (2010) who reported that N volatilization and denitrification affect soil TN. The interactions of the slope positions and locations were found to significantly ($p < 0.05$) affect the soil TN concentration (Table 3). There were significantly ($p < 0.05$) variations in the cation exchange capacity (CEC) across slope positions (Table 3).

The middle slope recorded the highest (18.10 cmol/kg) mean CEC while the lower slope had the lowest (14.7 cmol/kg) CEC. Across locations, Amaeze had the highest (20.2 cmol/kg) CEC followed by FCAI (19.4 cmol/kg) while the Fallow lowland had the lowest (11. 6 cmol/kg) CEC. Generally, the CEC in all these locations is within the range of moderate to low and high to low according to Landon (1991) and FAO (2006) standards respectively. The low CEC associated with the study locations suggests that the farmers do not apply adequate amount of organic manure to restoring the productivity the soil. Gachene et al. (2004) and Obalum et al. (2012) suggested that except gully cities, where urgent intervention may be needed, a cover cropping agronomic system can go a long way to conserving the “yet-to-be-degraded” soils.

CONCLUSION

Across the study locations, slope positions significantly affected the soil carbon stock due to the farming systems practiced by farmers in those locations. Significant differences in the chemical properties along slope positions (upper, middle and lower) were also observed. Soil pH and CEC were higher at the middle slope position; TN was significantly higher at lower slope position while SOC was significantly higher at upper slope position.

Overall, the study locations were associated with low SOC, CEC and soil nutrients. This suggests that the farmers do not apply sufficient amount of organic amendment, which is crucial to preventing soil erosion and nutrient losses, as well as enhancing crop productivity and improving the overall soil health.

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Soil Contamination and Sustainability of Open Market in Ihiagwa, Imo State, Nigeria

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Abstract:
The open market system presently adopted in Ihiagwa and across sub-Sahara Africa without proper management of market waste is causing serious concern to environmentalists and society. The case of Ihiagwa is peculiar, due to the high population density resulting from the presence of three big Federal institutions within and around Ihiagwa. In this study, three (3) soil samples were collected from 0-20 cm depth following the direction of natural drainage obtained by drainage analysis. Inversion resistivity measurement conducted showed subsurface lithology to the depth of the water table as shallow (11m) which indicates highly vulnerable. The soil samples were subjected to physiochemical and metal content analysis using standard analytical techniques. The physiochemical analysis of the soil samples revealed organic matter (OM), nitrogen concentration, phosphate, potassium and sodium (Na) to be above the FAO permissible limit, and significant levels of Pb, Zn, Cu and Iron giving a mean of 3.11mg/100g. Soil pollution load index (PLI) is >1 which indicates pollution. The overall analysis strongly confirms that the open market system in Ihiagwa is not environmentally friendly and not a sustainable practice in the area. The lack of waste management and population pressure has subjected the market to serious environmental stress calling for alternative markets such as establishing private shopping malls and plazas in the area.

Keywords: Open Market, Waste Dumping, Environmental Degradation, Soil Pollution, Sustainability.

INTRODUCTION

The ripple effect associated with waste dumping in Nigeria is underemphasized. According to Ozoh et al., (2021), indiscriminate waste dumping in most urban regions of Nigeria is heavily noted in market areas. These include wastes from several food products sold in such markets, animal wastes, human wastes and equipment wastes. Food poisoning, traffic congestion, aesthetic degradation, reduction in quality of life, and blockage of drainages are some of the problems caused by the lack of efficient waste management practices in these areas (Nwigwe, 2008). Studies conducted by Nwachukwu et al., (2010), also revealed a significant contamination of shallow wells (37 to 67 m deep) from the Owerri west flank of the Imo River basin of Nigeria due to indiscriminate dumping of waste close to urban settlements, lack of good toilet facilities, and transit grazing of cattle.

Solid waste disposal is treated with levity in Nigeria, which may be attributed to the low level of appropriate waste disposal sensitization amongst the public. The nonchalant attitude of people in Nigerian cities towards the health implications of indiscriminate waste disposal has posed a serious challenge to the adoption of modern waste disposal strategies in these areas (Afangideh et al., 2012). The refuse dumps remain in the surroundings of sellers for a long time, decomposing with strong stench, blocking the roads, leaching into groundwater systems and preventing ease of movement in and out of the market. In many nations of the world today, people, industries and local governments have polluted rivers, streams, and lakes through dumping of waste materials (Ogunbameru and Rotimi, 2006). Consequently, the dumping of refuse on the land leads to environmental pollution in the form of air, water and land pollution. This is because land pollution in the form of refuse and sewage produces an offensive odor and an ugly sight. This affects the oxygen from the air and perhaps accounts for one reason why air pollution according to the European Public Health Alliance (EPHA, 2009) is one of the most common forms of pollution throughout the world. Again, poor refuse and sewage disposal contaminates the well through dirty flowing water and pollutes the water with which meals are prepared in market restaurants and the water intake of individuals. The heaps of dirt further prevent the flow of water into other wells thus discouraging the digging of additional wells and making available water unsafe for drinking.

The lack of adequate management of refuse dumps which has contributed to the increasing pollution of the environment is still a clog in the wheel of environmental development in Owerri-west Local Government Area of Imo State (Taiwo and Ajayi, 2013). (Ugorji et al., 2020) reports open dumping of waste as the main waste disposal method, which is not only a source of environmental pollution but has become less adaptable in a town where land is becoming scarce due to population increase and high demand for physical development. The consequences of which are evident in stench and offensive smell, dirtiness of the environment and disease infestation. These pollute the air people breathe and adversely affect their health. Also, the source of water supply which is mostly wells in the market which are also polluted with the water that flows in from dirty drainages and water from animal dung are used to cook foods in the market which most of the marketer eat in their restaurants. All these can predispose marketers and their wards to waterborne diseases like typhoid, dysentery, diarrhea, cholera and other physical discomforts such as stench and aesthetic deprivation. It is against this backdrop that the study seeks to examine the impact of waste dumping on soil properties in the Ihiagwa market, Owerri West Local Government Area of Imo state.

MATERIALS AND METHODS

Study Area

Ihiagwa, situated in the Owerri West Local Government Area of Imo State, Nigeria, is a town located to the south of Owerri, the state's capital, at a distance of 12 km (7.5 mi) with a land area of 44,904 m^2 and a population of more than 20,000 (Nwachukwu *et al*., 2012a). It consists of eight distinct villages: Umuelem, Umuchima, Mboke, Nkaramochie, Iriamogu, Aku/Umuokwo, Ibuzo, and Umuezeawula. Ihiagwa has been split into two self-governing communities: Ihiagwa Ancient Kingdom (Chimelem), encompassing Umuelem and Umuchima; and Dindi-Ihiagwa, including the remaining six villages.

Ihiagwa is a community hosting the Federal University of Technology Owerri (FUTO) and in close proximity with two other Federal institutions located at its neighboring communities Nekede and Obinze precisely. The three Federal establishments are situated in close proximity, resulting in shared neighboring or host communities. These two institutions collectively accommodate a growing student population of over 35,000 individuals and a staff contingent exceeding 6,000 individuals (Nwachukwu *et al*., 2012a). This expanding population of students and employees is placing considerable strain on the host and nearby communities due to increased urbanization and improper waste management strategies (Nwachukwu *et al*., 2012a). A significant portion of students reside off-campus, while a substantial number of staff members seek lodging in the vicinity due to the considerable distance between the institutions and the city of Owerri or Owerri urban center. The surrounding area comprises four sizable communities: Ihiagwa, with an approximate population of 23,000; Nekede, with around 20,000 residents; Eziobodo, hosting about 10,000 people; and Obinze, with a population of roughly 15,000 individuals (Nwachukwu *et al*., 2012a).

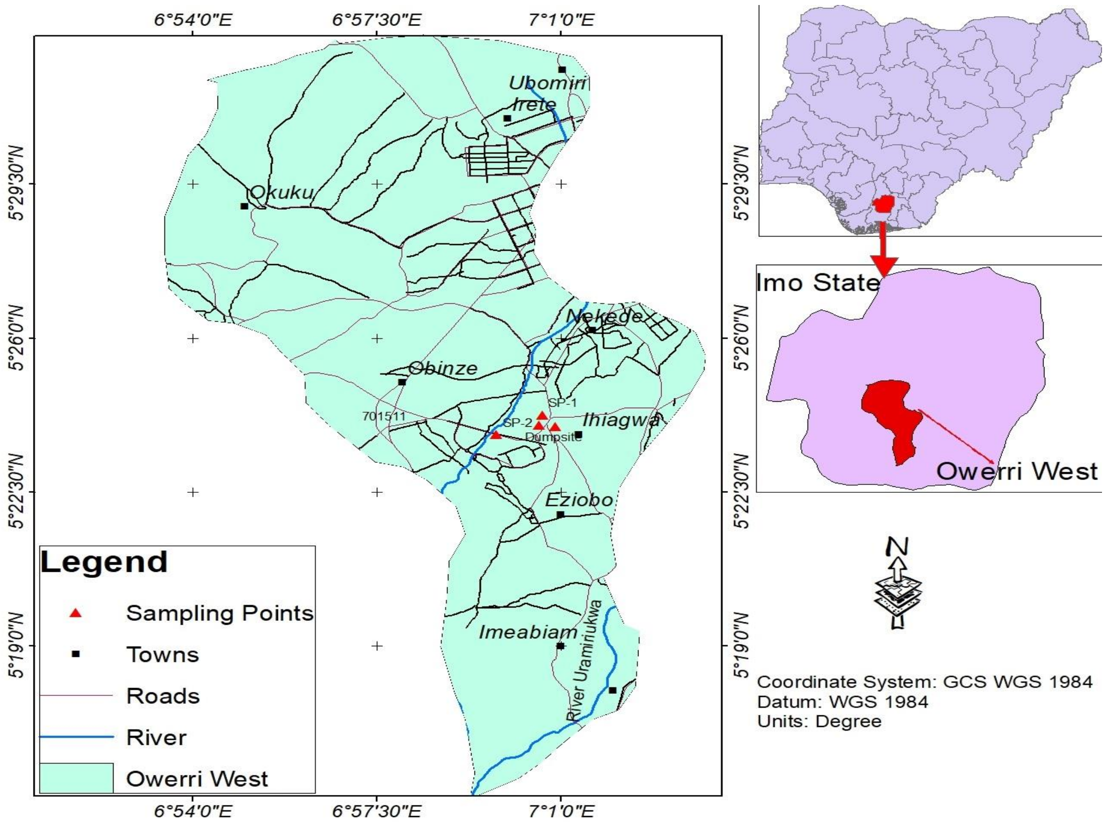

Fig. 1: Study Area Showing Sampling Points

Collection of Samples

Soil samples were collected from a total of three different locations using a hand auger from a depth of 15mm down the soil profile in an all the location and mixed to form a single sample method known as the composite method. This method of soil collection was done for the three different locations (market square, health center and Nkaramoche) in Ihiagwa. The soil samples were put inside a well labeled polythene bag for easy identification and sent to the laboratory for the required physiochemical analyses. The soil samples were air dried and stored at room temperature for laboratory analysis. The physiochemical parameters analyzed were; pH, % organic carbon, % organic matter, % nitrogen, available P, exchangeable acidity, exchangeable calcium, exchangeable Mg, exchangeable potassium, Magnesium, exchangeable Sodium, Iron, Copper, Zinc, % sand, % silt and % clay.

Determination of Parameters

The soil pH was carried out using a Glass-electrode pH meter. This was determined by preparing 1:2.5 soil to water ratio. 20g of air-dry soil was weighed into a 50ml beaker. 20ml of distilled water was added and allowed for 30 minutes. The mixture was stirred occasionally using a glass rod. Electrodes of the pH meter were inserted into the partly settled suspension and the pH was measured and reported as pH in water. The pH meter was calibrated with pH 7.0 and pH 4.0 buffer before use. The same procedure was carried out on 0.01 M $CaCl_2$ at 1:1 ratio of soil solution and 1N KCl ratio 1:2.5 soil to solution ratio.

The organic carbon was determined using the Walkley-Black method. Soil samples were weighed out in duplicates and transferred to 250ml Erlenmeyer flask. 10ml of 1N $K_2Cr_2O_7$ solution was pipetted into each flask and swirled gently to disperse the soil. 20ml concentration of H_2SO_4 was rapidly added using an automatic pipette. The flask was swirled until soil and reagent are mixed and allowed to stand for 30 minutes. 100ml of distilled water was added, 3-4 drops of indicator were added and titrated with 0.5N ferrous sulfate solution. The solution changes to dark green. Ferrous sulfate drops were added until the colour changed from blue to red. This formula was used to calculate:

$$\% \text{ Organic C in soil (air dry basis)} = \frac{(\text{me } K_2Cr_2O_7 - \text{me } FeSO_4) \times 0.003 \times 100 \times (f)}{\text{g of air-dry soil}}$$

Correction factor, f = 1.33
me = normality of solution x ml of solution used
% Organic matter in soil = % Org. C x 1.729

The total nitrogen was determined using Regular Macro-Kjeldahl Method. 10g of soil sample was weighed in a dry 500ml Macro-Kjeldahl flask. 20ml of distilled water was added, stirred and allowed to stand for 30 minutes. 1 tablet of mercury catalyst and 10g of K_2SO_4 was added. Also, 30ml of concentrated H_2SO_4 was added through an automatic pipette. The mixture was boiled for 5 hours. The flask was allowed to cool and 100ml of water was added to the flask. The sand residue was washed with 50ml of distilled water four times and transferred into the same flask. 50ml H_2BO_3 indicator solution was added into a 500ml Erlenmeyer flask. The 750ml kjeldahl flask was attached to the distilled apparatus and 150ml of 10N NaOH poured through the distillation flask to commence distillation. The condenser was cooled below 30°C. 0.01N standard HCl was titrated using 25ml burette at 0.1ml intervals. The colour changed from green to pink. The %N content in soil was then calculated.

For available phosphorus (P), 5ml aliquot of the soil extract was pipetted (Olsen extracts) into 25ml volumetric flask and 10ml of distilled water was added. 4ml of reagent was added to make up to volume distilled water. The colour was allowed to develop for 15 minutes and the P content in solution was determined on a spectrophotometer at 882 mμ.

For Exchangeable Potassium (K), Sodium (Na) and Calcium (Ca), a flame photometer was set up and instrument calibrated using standard solutions. The meter was set at zero while aspiring distilled water of blank solution. The meter reading was set at 100% E (emission) while aspiring the top concentration of standards. The % E reading was recorded at intermediate standard solution and plotting standard curve on linear graph paper. The % E reading was recorded checking 0 and 100% E reading with blank and top standard after every 10 to 20 sample determinations. The concentration of the element in sample solution was read from the standard curve and K, Na and Ca contents in soil was calculated.

For Magnesium (Mg), Copper (Cu), Zinc (Zn) and Calcium (Ca), The Perkin-Elmer Model instrument was set according to the PE 403 Atomic Absorption Spectrometer. The concentration readout was standardized using the standard solutions of the element tested and the concentration of the element in sample solution was read.

Contamination Factor and Pollution Load Index

To assess the level of contamination in the soil, the contamination factors (Cf) and pollution load index (PLI) are calculated and computed using (Esshaimi *et al*., 2012; Forstner and Calmano 1993) presented in equation 1 and 2.

$$CF = C_{\text{heavy metal}}/C_{\text{background}} \quad \ldots\ldots\ldots \quad (1)$$

$$PLI = (C_f1 * C_f2 * C_f3 \ldots\ldots\ldots Cfn)^{1/n} \quad \ldots\ldots\ldots \quad (2)$$

Where CF is the ratio obtained by dividing the concentration of each metal in the soil by the background value (Eq. 1) and n is the number of metals considered in the study.

Statistical Analysis

Statistical analysis of the data was by a TWO-WAY analysis of variance (ANOVA) without replication with an alpha value of $p<0.05$ for significance. Related Parameters (Heavy metals and physiochemical) were categorized, and then analysed to show significant degree of differences between the sample sites.

RESULT AND DISCUSSION

Table 1: Physiochemical Parameters of Soil Samples

PARAMETER	UNIT	SAMPLE 1	SAMPLE 2	SAMPLE 3	Mean	FAO
Coordinates		**$5^0 24^1 13^{11}$N $7^0 0^1 45^{11}$E**	**$5^0 24^1 9^{11}$N $7^0 0^1 39^{11}$E**	**$5^0 24^1 24^{11}$N $7^0 15^{11}$E**		
PH in water (1:25)		7.40	7.99	5.73	7.04	6.5-8.5
% Organic Matter		5.528	3.307	2.201	3.67	3
EC	us/cm	305	301	220	275.33	300-500
% Nitrogen		2.348	1.170	0.226	1.248	0.1-2.0
Available P	(Ppm p/g)	20.93	21.07	12.04	18.01	10-20
K	mg/100g	8.37	6.08	5.39	6.61	0.3-0.5
Na	mg/100g	5.27	5.65	7.14	6.02	0.3-0.5
Fe	mg/100g	3.425	3.117	2.788	3.11	0.05
Mn	mg/100g	2.855	2.763	2.429	2.68	NS
Cu	mg/100g	2.831	2.415	1.328	2.19	2
Zn	mg/100g	2.174	1.768	1.565	1.83	8.6
Mg	mg/100g	4.05	1.10	0.36	1.83	NS
Pb	Mg/100g	0.002	0.001	0	0.001	0.001
Exchangeable acidity	(cmol/kg)	5.16	0.60	1.12	2.29	NS
Exchangeable Ca		30.60	20.60	4.00	18.4	10-50
Exchangeable Mg		3.05	0.36	1.10	1.50	<5
Exchangeable K	(mg/100g)		0	0	0	
Exchangeable Na			0	0	0	
% sand		97.66	95.68	87.68	93.67	
% silt		2.0	2.00	8.00	4.00	
% clay		0.32	2.32	4.32	2.32	

EC= Electrical conductivity, K = Potassium, Na = Sodium, Fe = Iron, Mn = Manganese, Cu = Copper, Zn = Zinc, Mg = Magnesium, Ca = Calcium, Pb = Lead, FAO= Food and Agricultural Organization

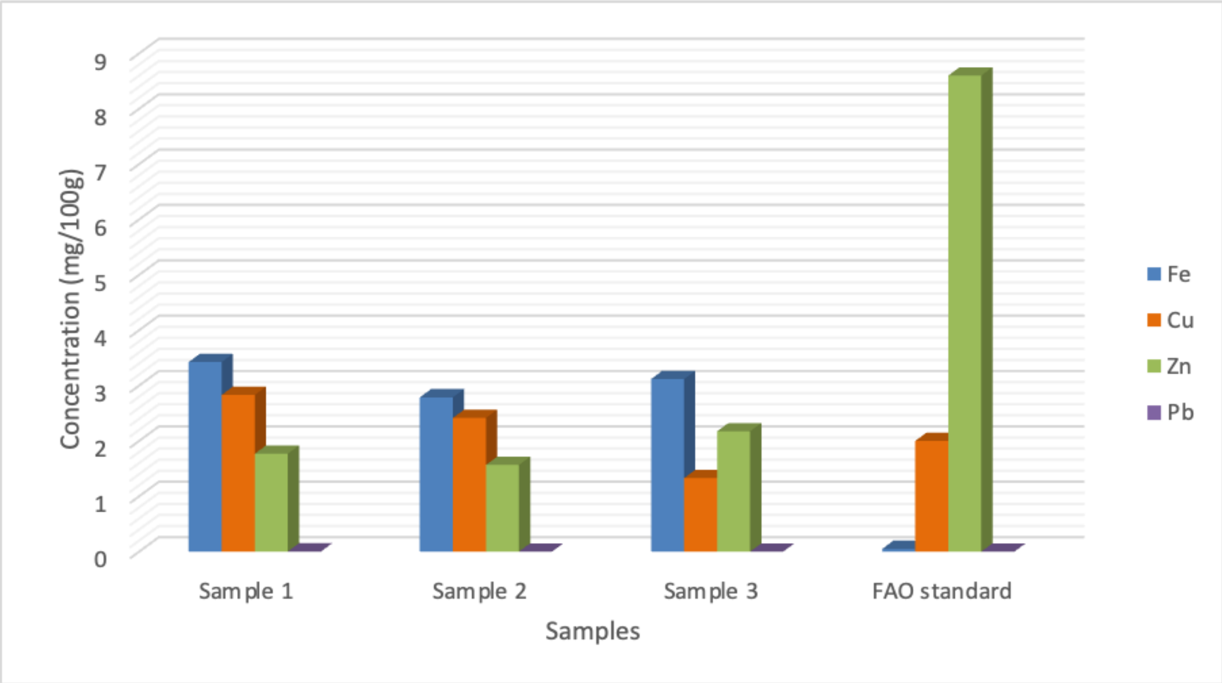

Figure 2: Heavy Metal Levels of Soil Samples Collected

Geophysical Result

The result acquired from the field was presented using the apparent resistivity values which was gotten from the automatic analysis of raw data gotten from the study area using the Advanced Geosciences Incorporation (AGI) ID software.

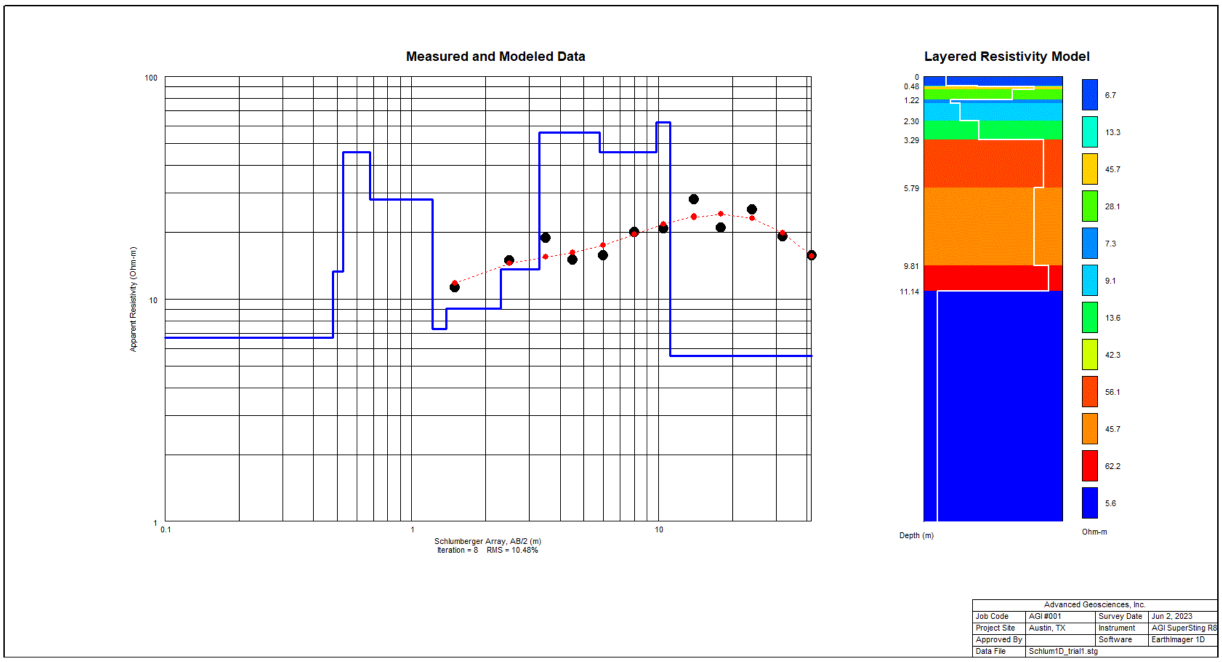

Fig 3: VES Model Result for Ihiagwa Market

Table 2: VES Analytical Result in Constrained Lithological Layers

Layer	Ohm-m	Depth (m)	Colour	Lithology
1	45.70	0.677	Brown	Topsoil (sandy)
2	7.34	1.382	Blue	Subsoil
3	42.25	3.300	Green	Subsoil
4	56.11	5.794	Red	Red earth
5	45.75	9.811	Orange	Silty sand
6	62.23	11.141	Light red	Siltstone
7	5.58		Blue	Saturated Aquifer unit

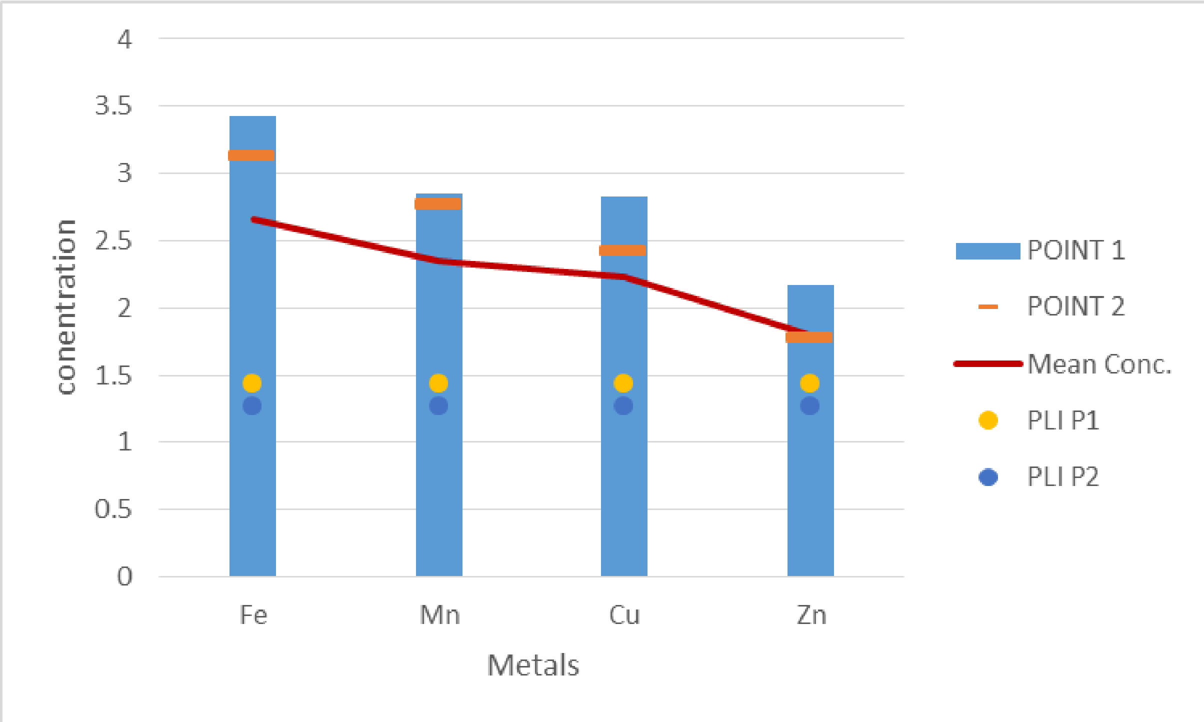

Fig 3.3: variability chart for PLI

Table 3: metal analysis and pollution load index

Metals	Point 1	CF point 1	PLI P1	Point 2	CF point 2	PLI P2	mean conc.	Point 3
Fe	3.425	1.228	1.438	3.117	1.118	1.272	2.66	2.788
Mn	2.855	1.175	1.438	2.763	1.138	1.272	2.352	2.429
Cu	2.831	2.132	1.438	2.415	1.819	1.272	2.228	1.328
Zn	2.174	1.389	1.438	1.768	1.130	1.272	1.793	1.565

The Ihiagwa market waste dumpsite contains various kinds of wastes from the environment and waste disposals; these are mainly organic wastes and food packaging materials. Result of Physiochemical and heavy metal analysis of the soil (Table 3.1) showed that the pH of the soil samples was below the permissible limit of 6.5 to 8.5 at sample 3 with a pH of 5.73, while sample 1 and 2 were within the permissible limits with a pH of 7.40 and 7.99 respectively. Organic natter (OM) was also above the FAO permissible limit of <3 at sample 1 and 2 with an OM of 5.528 and 3.307 respectively, while sample 3 recorded a 2.201 OM. Electrical conductivity (EC) of the samples ranged from 220µs/cm to 305µs/cm which was within the FAO permissible limit of 300-500µs/cm. Nitrogen concentrations in the soil ranged from 0.226% to 2.348% with sample 1 slightly above the FAO permissible limit of 0.1-2.0%. Available Phosphorus ranged from 12.04ppm/g to 21.07ppm/g with sample 1 and 2 above the FAO permissible limit of 10-20ppm/g. Potassium levels were all above the FAO permissible limit of 0.3-0.5mg/100g with the three samples reaching 8.37mg/100g, 6.08mg/100g, and 5.39mg/100gsd. High K concentrations in the soil solution inhibit Mg uptake and may induce Mg deficiency in plants (Trankner *et al*., 2018). Na levels were also higher than the FAO permissible limits of 0.3mg/100g – 0.5mg/100g for the three locations with 5.27mg/100g, 5.65mg/100g, and 7.14mg/100g for samples 1, 2 and 3 respectively. Root exposure to high sodium concentrations causes wilted foliage and stunted plant growth, which would impact negatively on the crop plants of the area.

Soil analysis conducted revealed the levels of various heavy metals in the soil such as Pb, Zn, Mn, Cu, and Fe in the Ihiagwa market waste dumpsite as shown in the result (Table 1). The levels of lead (Pb) in the soil were above the FAO and FME permissible standards with sample 1 at 0.002mg/100g, and sample 2 at 0.001mg/100g. Statistical analysis conducted for heavy metals on all samples showed significant levels of Pb with $p < 0.01$. The magnesium concentration of the soil was within the FAO and FME permissible limits of <5mg/100g. Zinc in the soil samples ranges from 1.565 mg/100g to 2.174mg/100g with an average mean of 1.835 mg/kg which were within the (FME, 2006) permissible limit of 5.00 mg/kg. The concentration of Cu contained in the three different soil samples ranges from 1.328mg/100g - 2.831mg/100g which were all above the FAO and FME permissible limits of <2mg/100g. Iron concentration in the soil samples is from 2.788mg/100g to 3.425mg/100g with a mean average of 3.11mg/100g. All the soil samples contain higher concentrations of iron when compared to the (FME, 2006) permissible limit of 1.00mg/100g which is a problem as Iron is toxic when it accumulates to high levels.

CONCLUSION

The investigation of the environmental impacts of waste dumping on soil and in Ihiagwa market has revealed that waste accumulated due to the improper waste dumping as disposal method affects the soil quality and is capable of altering the composition of the environment. The heavy metal pollution level in the study area can be linked to the indiscriminate dumping of waste in the area as the metal pollution is not of natural decomposition.

The environmental quality assessment in this study is based on a comparative analysis of soil. Based on soil contamination and pollution index, open market degrades the environment and therefore establishment of open markets without proper environmental management approaches should be discouraged. The pollution index load values also showed significant pollution level in determining the heavy metal concentration. This indicates presence of heavy metal pollution in the soil of the study area. This causes deterioration of the soil quality attributed to the dumping of waste directly on the soil surface as contaminates easily penetrate and contaminate the soil posing potential risk on the soil.

The physiochemical analysis results obtained from the soil sample revealed organic matter, nitrogen concentration, phosphorus, potassium and sodium were above the FAO permissible limit. Soil analysis conducted also revealed presence of heavy metals in the soil such as lead, zinc, magnesium, copper and iron.

The result obtained from the analysis has revealed the presence of different contaminates in the study area which contributes to environmental problems associated with improper waste disposal method and waste management. This confirms that the practice of open market system without waste management system is not environmentally friendly and not a sustainable practice and as such poses potential risk to soil.

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Fields Preparation Techniques for Rice Production: 2: Impacts on Carbon Sequestration, Soil pH and Grain Yield of Lowlands Rice in Southeastern Nigeria

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Abstract:
An inland valley of Federal College of Agriculture, Ishiagu, Ebonyi State, research farm was used in 2018 and 2019 cropping seasons to determine the impacts of fields' preparation techniques on carbon sequestration, soil pH and grain yield of rice in Ishiagu. A randomized complete block design was employed for the study. Field preparation techniques employed include; Cleared, tilled, seedlings-transplanted and hand weeding (C/T/ST//HW); Non-selective herbicide applied, cleared, tilled, seedlings-transplanted and herbicide for weeding (N-H/C/T/ST); Applied non-selective herbicide, cleared, broadcasted seeds and herbicide for weeding (N-H/C/B); Applied Non-selective herbicide, broadcasted and herbicide for weeding (N-H/B); Cleared, tilled, seedlings-transplanted and herbicides for weeding (C/T/ST/S); Applied Non-selective herbicide, cleared, tilled, seedlings-transplanted and hand weeding (NHw/ C/T/ST/Hw). Results showed that C/T/T/HW sequestrated soil carbon higher (37.02 g/m2) than other field techniques in the 1st year, as N-H/C/B and N-H/C/T/T gave the highest improvement in the 2nd year. Soil organic carbon (SOC) improvement (1.3443%) in the 1st year was obtained from C/T/T/HW plots, whereas in the 2nd year, N-H/C/B plots gave the highest (1.5070%) significant ($p < 0.05$) increase. Results revealed that C/T/T/HW plots increased pH higher (5.13) in the 1st year, while in the 2nd year, N-H/C/T/T gave the highest (5.40) improvement. Findings from this study showed that soil carbon sequestration can be greatly enhanced by the type of rice field management practice(s) adopted during planting and at the time of weed control. It is therefore concluded that, since herbicides application is the most common practice and cheaper approach in the study area, farmers are recommended to use non- selective herbicides to clear the vegetation but use manual weeding operation subsequently in the control of weeds with proper water management which will help in weed control in their rice fields.

Keywords: Herbicides, rice grain yield, carbon sequestration, broadcasting, transplanting

INTRODUCTION

The production of lowland rice in most rice producing areas in Ebonyi State is rain-fed dependent (Nwite *et al.*, 2013 and 2017); and application of non- selective herbicides to clear the vegetation and subsequent broadcasting of rice seeds (In-situ) is the predominant land preparation techniques in the study area. In most cases, farmers do not adhere to manufacturers' manuals or instructions during the application of these herbicides leading to misuse and inappropriate applications. This agricultural or farming practice has led to variations in the crop yield, soil organic carbon build and ecological changes. Farmers in the study area do not have environmental risk awareness of using wrong farming practices; they adopt non-sustainable farming practices such as the extensive use of herbicides instead of mechanical or cultural method of weed control in their rice fields, thus affecting soil organic carbon characteristics and fertility status (Malamataris *et al.,* 2023). Traditional field technique for water management systems in the lowlands rice production in Ebonyi State that is regarded as a major rice producing State in Nigeria are characterized by the fact that farmers focus on storage of water in the rice field, without any possibility to divert water from one place to another. Poor soil fertility and inefficient weed and water control are the major constraints to proper utilization of these inland valleys for sustainable rice-based cropping (Nwite *et al.,* 2013).

The soils of the studied area are known in decades past to have a high potential for carbon sequestration because soil organic carbon content can be conserved, restored and increased through appropriate land uses and agricultural management practices that can be applied at the landscape level (Corsi *et al*., 2012), but the current land uses and agricultural management practices in the area is gradually facing out, hence, affecting the soil health and potentials.

Soil organic carbon (SOC) sequestration is mainly influenced by such key factors as the amounts of carbon input (Bahman and Ginting, 2003), plant residue management (Thelen *et al*., 2010), soil depth (Blanco-Conqui and Lal, 2007) and soil texture (Gami *et al*., 2009). Storage of SOC in agricultural systems is a balance between carbon additions from non-harvested portions of crops (Wu *et al*., 2008), organic sources (Thalen *et al*., 2010), and carbon losses, primarily through organic matter decomposition and release of respired CO_2 to the atmosphere.

Usui and Kasubuchi (2011), submitted that after herbicide application, the diurnal variation in CO_2 concentration was reduced, and the minimum concentration of CO_2 increased. They went further to state that the high CO_2 concentration during the daytime and the reduction in variation after herbicide application were caused by a decrease in CO_2 consumption owing to the inhibition of photosynthesis. The herbicide application could lead to changes in CO_2 concentration by the reduction of cyanobacteria and microalgae (Usui and Kasubuchi, 2011).

Usui *et al.* (2003) reported that CO_2 concentration is related to pH in ponded water and that comparing pH with RpH (the pH at which the CO2 concentration of the water is in equilibrium with that of the air) confirmed whether the water had absorbed or emitted CO_2.

The study aims at evaluating different field techniques in inland valley rice field as they affect carbon sequestration, pH of the soil and grain yield of rice.

MATERIALS AND METHODS

The study was carried out at the inland valley rice field at Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria, during the 2018 and 2019 cropping seasons. The field was under rice cultivation by the Institution before the study. The area is located within latitude 05^{o} 56′ N and longitude 07^{o} 41′E. The mean annual rainfall and mean monthly temperature have been reported as 1350 mm and 30^{o} C respectively. The area lies within the derived savanna vegetative zone of South Eastern Nigeria. There are two reported distinct seasons, the dry season which spans November to March, at times extend to April, and the rainy season which spans April to October (Nwite *et al.*, 2008).

Geologically, the area is underlain by sedimentary rock derived from successive deposit of the cretaceous and tertiary period and lies within Asu River Group (Lekwa *et al.,* 1995). The location lies within the Asu River Group and consists of Olive brown sandy shale, fine grained micaeous sandstones and mudstones deposited in an alternating sequence. Generally, Ebonyi State lays mostly in the Ebonyi (Aboine) river Basin and Cross River plains. The area contains two main geological formations. The soil is hydromorphic and belongs to the order Ultisol. It has been classified as typic Haplustut (FDALR, 1985).

Agronomic Practices

Land Preparation:

The land used was prepared according to the specifications outlined in each treatment. The plot size measured 2.6 m x 3.4 m.

Rice fields preparation techniques employed for the study and their descriptions include the following;

- Clear, till and transplant + use of hand weeding (C/T/T/HW). The plots involved were cleared, tilled manually with native hoe, and followed by transplanting. Hand weeding was adopted during weeding operation.
- Non-selective herbicide was applied, cleared, tilled and transplanted + use of herbicide for weeding (N-H/C/T/T). A branded name non-selective herbicide (Uproot) was applied to kill the grasses in the affected plots; after two weeks of application, the plots were cleared of the dead grasses, later tilled with native hoe and rice seedlings transplanted. During the weeding operation, a branded name – orizo plus selective herbicide was applied in the designated plots.
- Non-selective herbicide (Uproot) was applied, cleared, broadcast + use of herbicide for weeding (N-H/C/B). A branded name non-selective herbicide (Uproot) was applied at the rate of 150 mls a.i./20 litres of water to kill the grasses in the affected plots; after two weeks of application, the plots were cleared of the dried grasses, re-touched with the same herbicide at the rate of 100 mls a.i/20 litres of water. This was followed by broadcasting of rice seeds at the rate of 40 kg/ha. Weeding of the plots was carried out using orizo-plus selective herbicide at the rate of 150 mls a.i./20 litres of water.
- Non-selective herbicide was applied and rice seeds broadcast + use of herbicide for weeding (N-H/B).
- Clear, till and transplant + use of orizo-plus selective herbicide for weeding (C/T/T/S). The plots involved were cleared, tilled manually with native hoe, and followed by transplanting. During weeding operation, orizo-plus selective herbicide was used for weeding.
- Non-selective herbicide was applied, clear, till and transplant + use hand weeding (N-Hw/C/T/T/ Hw). A branded name non-selective herbicide (Uproot) was applied to kill the grasses in the affected plots; after two weeks of application, the plots were cleared of the dried grasses, later were tilled with native hoe and rice seedlings transplanted. Hand weeding was used during weeding operation in the affected plots.

Inorganic fertilizer (NPK 20:10:10) combined with rice husk dust was applied basally at 200 kg/ha and 5 t/ha, respectively, in all the plots. The rice husk dust was applied to those plots where seeds broadcasting was carried out at the time of the broadcasting, while the rice husk dust was applied and incorporated to the tilled and transplanted plots at the period of tillage operation. The NPK 20:10:10 was applied two weeks after germination or two weeks after transplanting as the case may be.

Planting:

- **Broadcasting method:** The test crop was rice (*Oryza sativa* –FARO 57 variety). The plots where rice seeds broadcasting was adopted as a method had no determined plant spacing but were thinned 2 weeks after germination where they were found to be crowded. The broadcasted rice seeds started germinating after 4 days. The plots were ponded with water after one month of germination.
- **Transplanting:** Nursery bed was established for these plots where rice transplanting method was practiced. In this regard, a raised bed was made, followed by broadcasting of rice seeds on the raised bed. The seed rate in transplanting is 30kg/ha as against 40kg/ha in broadcasting method. Prepared nursery was ready for transplanting into field at the age of 3 weeks, as transplanting of seedlings at later age reduces tillering rates. Transplanting was done by uprooting the seedlings and washing-off the attached soil particles on the roots and transplanted with the aid of fork-sticks. Planting space of 20 cm x 20 cm was used in the transplanted plots to give a plant population of 221 stands/plots and a total of 250,000 stands/ha.

Weeding:
Weeding operation was carried out manually by hand or with herbicides according to the treatments' specifications at the right time.

Soil Sampling and Laboratory Analyses
Bulk soil samples were collected from 0 to 20 cm depth using soil auger and core samples were also collected before the field preparation specifications for pre-planting soil analysis. Similarly, after each cropping season (after harvest), another set of auger and core soil samples were taken from individual plots. The auger samples were air-dried and sieved through 2.0-mm mesh and taken to laboratory for particle size distribution, soil pH and organic carbon analyses. The soil pH was determined in distilled water and 0.1 N KCl solution using a soil solution of 1:2.5 (Mclean 1982). SOC was determined by wet oxidation method (Walkley and Black 1934) as modified by Nelson and Sommers (1996). Particle size distribution of the samples was determined by the hydrometer method (Gee & Bauder 1986). The core samples were allowed to drain freely for 24 h before being oven dried for determination of bulk density by the Blake and Hartge's (1986) method.

Statistical Analysis
Data collected were analyzed using GENSTAT 3 7.2 Edition. Significant treatment means were separated and compared using Fisher's least significant difference (F-LSD) at 5% probability level according to Obi (2002).

RESULTS AND DISCUSSION

Initial Soil Characteristics of the Studied Soil (0 – 20 cm depth)
Table 3.1 indicated that the textural class of the studied soil is sandy clay soil with percentage level of clay as 200 g/kg, 230 g/kg silt and 530 g/kg fine sand content, while the coarse sand value stands as 40 g/kg. The results also showed organic carbon percent as 1.14% as total nitrogen and pH of the soil recorded 0.84 % and 4.8, respectively. The soil carbon sequestration and initial soil bulk density of the area is 18.84 g/m^2 and 1.64 Mg/m^3.

Table 3.1 Initial soil characteristics of the studied soil (0 – 20 cm depth)

Soil Properties	Values
Clay (g/kg)	200
Silt	230
Fine sand	530
Coarse sand	40
Textural class	Sandy clay
Organic carbon (%)	1.14
Total nitrogen (g/kg)	0.84
pH (H_2O)	4.8
Bulk density (Mg/m^3)	1.68
Carbon sequestration (g/m^2)	18.84

Effects of Rice Fields Preparation Techniques on Carbon Sequestration and Soil Organic Carbon

Results in Table 3.2 revealed that there were significant ($p < 0.05$) variations on soil carbon sequestration due to the different field techniques studied. It was recorded that C/T/T/HW (clear, till and transplant + hand weeding) with a value 37.02 g/m^2 sequestrated soil carbon higher than other field study within the 1st year of study. It was obtained that in the 2nd year, it was N-H/C/B (Apply non-selective herbicides, clear and broadcast + selective herbicide) and N-H/C/T/T (Apply non-selective herbicides, clear, till and transplant + selective herbicide) that gave the best significant improvement on the carbon sequestration of the studied soil.

The lowest improvement on the soil carbon sequestration in the 1st year was recorded in plots with N-H/B, N-H/C/B, N-H/C/T/T and N-H/C/T/T/Hw as their values are statistically same and lower from other field techniques studied. Generally, there were significant increasing variations on the carbon sequestration among the different field techniques studied except in plots with C/T/T/HW system where the carbon sequestration decreased from 37.02 g/m^2 in the first year to 29.82 g/m^2 in the second year of study.

Soil organic carbon (SOC) was shown to have been significantly ($p < 0.05$) varied in both first and second year of study (Table 3.2). The results showed that best significant ($p < 0.05$) soil organic carbon improvement (1.3443%) in the 1st year was obtained from plots with C/T/T/HW, while N-H/C/T/T/ Hw (non-selective herbicides, clear, till and transplant + hand weeding) with value of 1.1067% recorded the least improvement. In the 2nd year, N-H/C/B plots gave the highest (1.5070%) significant ($p < 0.05$) increase on the soil organic carbon as plots with N-H/C/T/T/Hw recorded the lowest (1.2227%) improvement on the SOC within the period. Generally, it was noted that there was a strong positive relationship or link between soil organic carbon and carbon sequestration especially in the 2nd year of study, as the same field preparation technique (N-H/C/B) gave the best significant ($p < 0.05$) increased soil carbon sequestration and organic carbon pools. Studies have shown that soil organic carbon (SOC) sequestration is mainly influenced by such key factors as the amounts of carbon input (Bahman and Ginting, 2003) and plant residue management (Thelen *et al*., 2010).

Table 3.2: Effects of rice fields preparation techniques on carbon sequestration and soil organic carbon

Treatments applied	Carbon sequestration (g/m-2)		Soil organic carbon (%)	
	Year 1	Year 2	Year 1	Year 2
C/T/T/S	31.45	32.42	1.1820	1.3037
C/T/T/HW	37.02	29.82	1.3443	1.1323
N-H/B	27.04	32.18	1.1393	1.3443
N-H/C/B	26.51	37.58	1.1423	1.5070
N-H/C/T/T	28.98	36.83	1.1157	1.3440
N-H/C/T/T/ Hw	26.59	30.65	1.1067	1.2227
Grand mean	**29.60**	**33.25**	**1.1717**	**1.3090**
F-LSD 0.05	**3.540 p = < .001**	**5.105 p = 0.031**	**0.03867 p = < .001**	**0.04197 p = < .001**

N-H/C/T/T = Apply non-selective herbicides, clear, till and transplant + selective herbicide; C/T/T/HW= clear, till and transplant + hand weeding; N-H/C/B = Apply non-selective herbicides, clear and broadcast + selective herbicide; N-H/B = Apply non-selective herbicides, broadcast+ selective herbicide; C/T/T/S = Clear, till and transplant + selective herbicide; N-H/C/T/T/Hw = non-selective herbicides, clear, till and transplant + hand weeding; F-LSD=Fisher's least significant difference

Effects of Rice Fields Preparation Techniques on Soil Bulk Density and pH (H_2O)

The results showed that bulk density of the studied soil did vary in the 1st year of study but did not change among the different field techniques in the 2nd year of study (Table 3.3). It was obtained that the highest significant increased bulk density of 1.377 Mg/m^2 was recorded in plots with C/T/T/HW in the 1st year, while the least reduction of BD was obtained in N-H/B plots with 1.187 Mg/m^2. Soil BD in the 2nd year was best reduced in the same N-H/B managed plots.

Soil pH was significantly ($p < 0.05$) influenced by the different field techniques in both 1st and 2nd year of study. It was obtained that in the 1st year, the most improved pH in water (5.13) was recorded in the C/T/T/HW plots, followed by C/T/T/S managed plots (5.03) while the least pH of 4.83 was obtained from N-H/B plot. In the 2nd year, N-H/C/T/T plots gave the highest (5.40) significant ($p < 0.05$) improvement on the soil pH, while the lowest pH (5.03) was obtained in plots with N-H/B field management.

Table 3.3: Effects of rice fields preparation techniques on soil bulk density and pH (H_2O)

Treatments applied	Bulk density(g/m-2)		Soil pH (H_2O)	
	Year 1	Year 2	Year 1	Year 2
C/T/T/S	1.330	1.243	5.03	5.33
C/T/T/HW	1.377	1.317	5.13	5.30
N-H/B	1.187	1.197	4.83	5.03
N-H/C/B	1.160	1.247	4.87	5.27
N-H/C/T/T	1.300	1.370	5.00	5.40
N-H/C/T/T/ Hw	1.200	1.253	4.87	5.20
Grand mean	**1.259**	**1.271**	**4.96**	**5.26**
F-LSD 0.05	**0.1494 p = 0.044**	**NS**	**0.0978 p = < .001**	**0.1435 p = 0.003**

N-H/C/T/T = Apply non-selective herbicides, clear, till and transplant + selective herbicide; C/T/T/HW= clear, till and transplant + hand weeding; N-H/C/B = Apply non-selective herbicides, clear and broadcast + selective herbicide; N-H/B = Apply non-selective herbicides, broadcast+ selective herbicide; C/T/T/S = Clear, till and transplant + selective herbicide; N-H/C/T/T/Hw = non-selective herbicides, clear, till and transplant + hand weeding; F-LSD=Fisher's least significant difference

Effects of Rice Field Preparation Techniques on Grain Yield of Rice

The rice grain yield in the first year was not significantly ($p < 0.05$) improved among the studied field preparation techniques. The results (Table 3.4) indicated that C/T/T/HW adopted plots gave the highest (5.89 t/ha) mean rice grain yield, whereas N-H/B plots recorded the lowest (3.93 t/ha) rice grain yield. The results (Table 3.4) revealed that plots with N-H/C/T/T/Hw yielded significantly ($p < 0.05$) higher (8.40 t/ha) of grains than other field techniques used, while N-H/B plots recorded the least (3.45 t/ha) grain yield within the 2nd year of study. It was generally observed that the second-year rice grain yield performed appreciably higher than the first year of the study, except in N-H/B fields where its yield reduced by 0.48 t/ha. It was recorded that in N-H/C/T/T/ Hw field method, the difference in yield between year 1 and year 2 of the study was 20.2% yield difference. Herbicides have remarkably reduced farming labor and contributed to an increase in labor productivity; therefore, a good understanding of the manufacturers' advice or instruction for each product must be strictly adhered to, to avoid overuse or misuse.

Table 3.4: Effects of rice field preparation techniques on grain yield of rice (t ha^{-1})

Treatments applied	**Rice Grain yield (tons/hectare)**	
	Year 1	**Year 2**
C/T/T/S	4.18	4.88
C/T/T/HW	5.89	7.66
N-H/B	3.93	3.45
N-H/C/B	5.54	5.60
N-H/C/T/T	5.07	5.87
N-H/C/T/T/ Hw	5.58	8.40
Grand mean	**5.03**	**5.98**
F-LSD 0.05	**NS**	**2.141**

N-H/C/T = Apply non-selective herbicides, clear, till and transplant + selective herbicide; C/T/T/HW= clear, till and transplant + HAND WEEDING; N-H/C/B = Apply non-selective herbicides, clear and broadcast + selective herbicide; N-H/B = Apply non-selective herbicides, broadcast+ selective herbicide; C/T/T/S = Clear, till and transplant + selective herbicide; N-H/C/T/T/Hw = non-selective herbicides, clear, till and transplant + hand weeding; F-LSD=Fisher's least significant difference

Relationship Between Soil pH and Carbon Sequestration

Figures 1 and 2 revealed that soil pH even though has positive influence on the soil organic carbon, but does not have stronger influence on the soil organic carbon ($R^2 = 0.495$ and $R^2 = 0.0017$) in the 1st and 2nd year, respectively. The results also showed that there was no strong relationship between the soil pH and carbon sequestration (Figures 3 and 4) for the two years of study. The soil pH showed no strong influence as it recorded $R^2 = 0.3153$ in the 1st year and $R^2 = 0.0672$) in the 2nd year of study. The implication of these results for rice fields' management of the inland valley soils for rice production and improved soil organic carbon, including the soil carbon sequestration is that while adopting a particular field preparation practice, priority attention should be on managing the soil pH. These results were in disagreement with the findings of Usui *et al.* (2003), who reported that CO_2 concentration is related to pH in ponded water and that comparing pH with RpH (the pH at which the CO2 concentration of the water is in equilibrium with that of the air) confirmed whether the water had absorbed or emitted CO_2. It could be noted that the difference between their findings and these results could be that their own work was on field ponded water as against the field soil undertaken in this study.

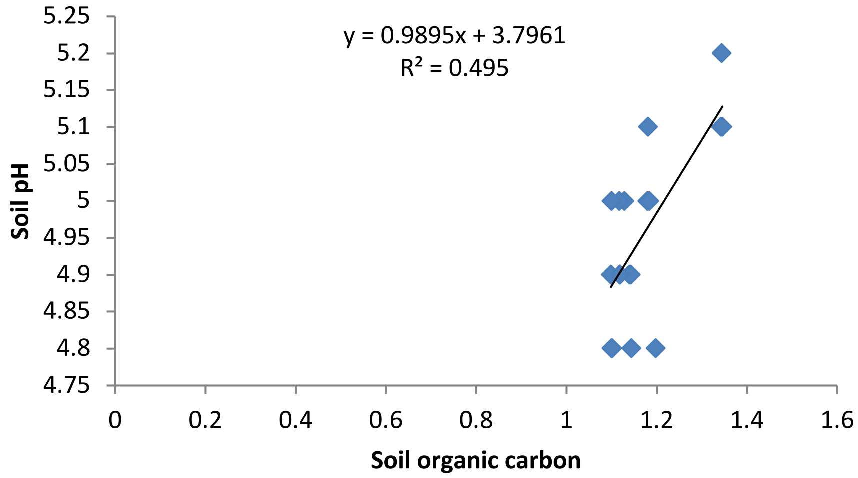

Figure 1: Soil organic carbon and pH relationship in yr 1

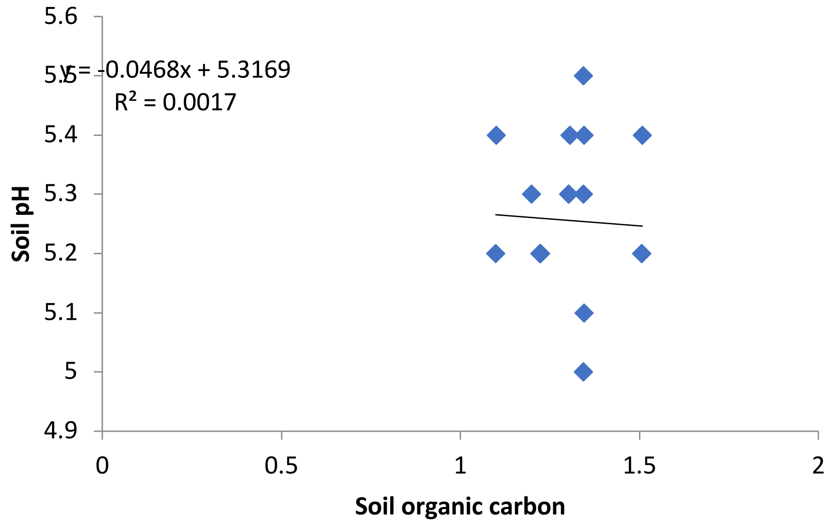

Figure 2: Soil organic carbon and ph relationship in yr 2

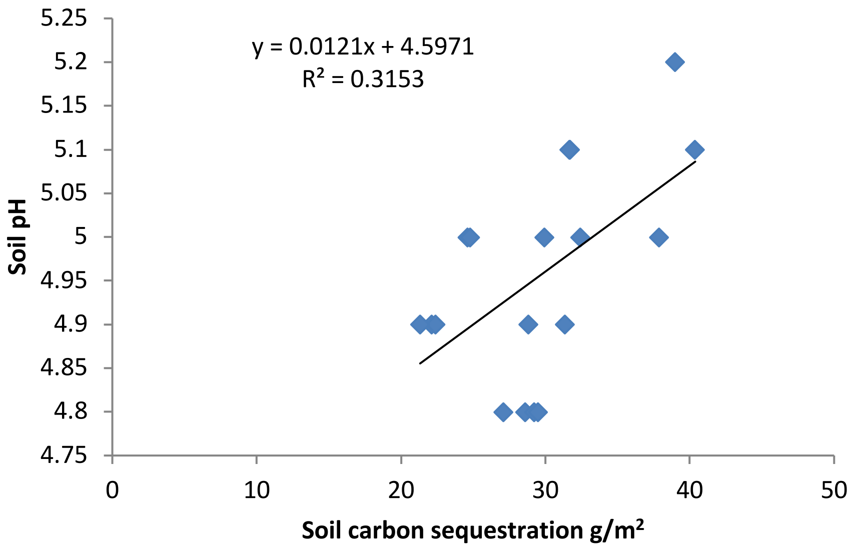

Figure 3: pH/carbon sequestration relationship yr 1

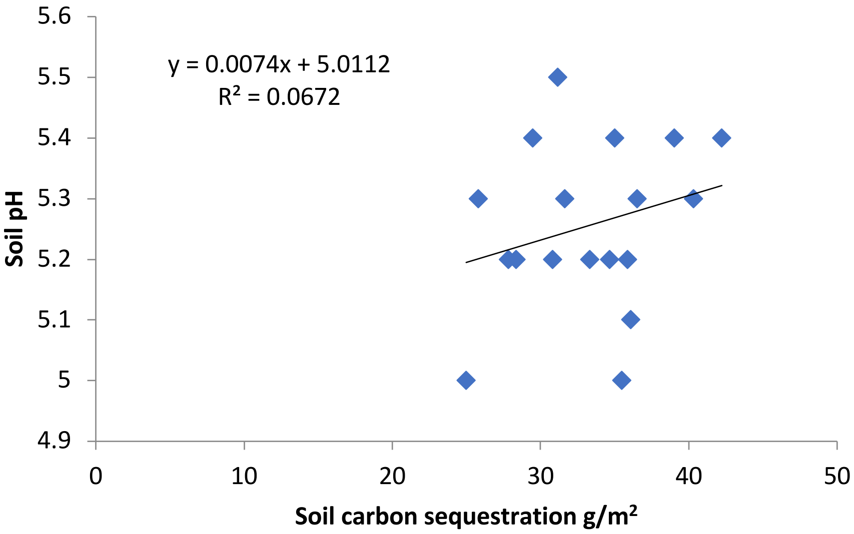

Figure 4: pH/carbon sequestration relationship yr 2

CONCLUSION

The findings from this study within the two years of study showed that soil carbon sequestration can be greatly enhanced by the type of rice field management practice(s) adopted during planting and at the time of weed control in the rice fields. It can be deduced that use of herbicides application as preparatory measure for planting and weeding increasingly influenced soil carbon sequestration in the study area. Applying non-selective herbicides, clear and broadcast plus the use selective herbicide (N-H/C/B) for weeding operation increased carbon sequestration in the 2nd year but increases soil bulk density unimaginably higher within the two years of study, though falls lower than the root limiting level.

The results revealed that soil organic carbon as well increased by the same field management practice in the 2nd year of study. However, soil pH was better appreciated in N-H/C/T/T adopted plots within the periods. Rice grain yield was best improved by N-H/C/T/T/Hw (non-selective herbicides, clear, till and transplant + hand weeding). The findings therefore revealed that constant use of herbicides in rice fields can drastically reduce rice grain yield and increase soil bulk density in the farmers' fields. Since herbicides application is the most common practice and cheaper approach in the study area, farmers are recommended to use non- selective herbicides to clear the vegetation but use manual weeding operation subsequently in the control of weeds with proper bunding for water management which will as well help in weed control in their rice fields.

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Growth and Yield Response of Marigold to Potting Media Containing Different Ratio of Manures

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Abstract:
The experiment was conducted at department of Horticulture, Sindh Agriculture University Tando Jam, Sindh, Pakistan, to evaluate the Growth and yield response of marigold to potting media containing different ratio of manures. The experiment was conducted in Randomized complete block Design (RCBD). The study comprised of five different potting media containing different ratio of manures treatments including T1= Soil (S)–control, T2 = 50% Farmyard manure + soil, T3 = 50% poultry manure + soil, T4 = 50% silt + soil, T5 = 30% Farmyard manure + 30% poultry manure + soil, Seedlings of marigold were transplanted in Pots. each treatment was replicated three times. The results indicated a significant effect of the treatments observations were recorded for plant height (cm), Width of leaves, number of flowers per plant, number of branches per plant, fresh biomass of flower (g), flower diameter (cm). It was found that all studied attributes were statically significant ($P<0.05$) and responded by potting media containing different ratio of manure. The maximum plant height (15.113 cm) was obtained from T5= 30% Farmyard manure + 30% poultry manure + soil, 30% Farmyard manure + 30% poultry manure + soil T5 produced maximum Number of flowers per plant (24.13) and maximum branches per plant (4.64), maximum fresh biomass of flower (210. .45 g) were observed From the T5= 30% Farmyard manure + 30% poultry manure + soil, followed by T4= 50% silt + soil. Observed (14.997 cm) Plant height, (23.94) flowers per plant, (4.54) branches per plant, and maximum and minimum plant height (10.304 cm), minimum number of flowers per plant (16.31), minimum number of branches per plant (2.88), minimum fresh biomass of flower (150.26 g), minimum flower diameter (10.34) cm, was observed from the T1 =Soil =Control-No manure.

Keywords: marigold, potting media, manure.

INTRODUCTION

Marigold is one of the famous flowers scientific Name: (Tagetes erecta L.) Common Name: Marigold) belongs to Asteraceae or Compositae family (Kumar, N., N. Kumar, J. P. Singh and H. Kaushik. 2016). Those flower use for many purposes like non secular / spiritual, birthday party / feature and maximum of the festivals. Marigold flora is to be had in attractive shapes and coloration and sizes. As a result, they're ideal for any lawn ornament or making garlands. Marigold is used both for ornamental and medicinal purposes. It is also used in cosmetic and perfume industry due to its aromatic nature and essential oil contents (Regaswamy, D. and J. Koilpillai. 2014)Because of a brief cropping duration and low funding and care made this flower to turn out to be popular amongst flower growers. In Pakistan, African marigold plant life is sold within the market as loose for making garland. Aside from reduce flower, marigold especially is used for beautification and additionally panorama flowers due to its variable peak and colorings of plant life. It's far surprisingly suitable as a bedding plant in an herbaceous border and is likewise perfect for newly planted shrubberies offer coloration and fill the spaces. (Arora et al.).

Marigolds have smaller flowers and leaves than most other marigolds. Plants decorate the sunny places of the landscape and attract attention. In addition, amaranth is a very valuable crop for fighting nematodes that parasitism on plant**s**, (Basu, S. D., Roy, S. K., 1975) The aboveground part of the plant contains high-quality essential oils that can be used for the aromatization of soaps in the perfume, cosmetic and pharmaceutical industries. Mycorrhizal symbiosis of arboreal mycorrhizal is widely believed to protect host plants from the harmful effects of drought (Rahbarl, M., Omidi, M. and Shahram, S. 2013). Increased water absorption at low soil moisture levels as a result of non-root hyphae. Marigolds are usually annuals that respond to fungal infections, but they do not always show a significant response under restricted conditions Upright Tagetes is the 3rd most important cut flower in the world market after roses and carnations. The flowers of this plant have different colors. They are in great demand and are especially appreciated on Easter and Mother's Day (Blondo, R. J. and Noland, D. A. 2000). It is recommended to achieve Erecta flowering (Acharya, M. M. and L. K. Dashora 2004). The plant has a longer flowering period, so it can be used in a variety of situations in the home garden (Golestani, M., Dolatkhahi, A. and Kazemi, F. 2013) Upright Tagete flowers can also be used zipal T sheets of natural yellow-orange pigment helenin (xanthophyll), which is in great demand among national and international companies (Ali, E. F. and Hassan, F. A. 2013). Elekta as a tea with spices (sitkovich et al. It was revealed that the two men had been involved in a series of incidents in which they had been involved in a series of incidents. Upright Tagetes can be used in different situations of home garden and landscape design. This is one of the best plants for planting in rock gardens, flower beds and balconies. In addition to having pesticide properties against nematodes and some pests common in the garden, this plant is also considered a snake repellent in the garden (Sasikumar, K., Baskaran, V., Abirami, K., 2015.). The choice of a good nutrient medium is the basis for the proper management of the nursery and the basis for a healthy root system. Nutrient media for use in container nurseries are available in 2 main forms: soil and organic systems (Hartmann et al. 2007) Compared with soil medium, its main component is field soil, organic medium (the basis of organic matter, which can be compost, peat, sawdust, rice husk, coconut, bird droppings, etc.).

MATERIAL AND METHODS

The experiment was conducted at department of Horticulture, Sindh Agriculture University Tando Jam, Sindh, Pakistan, to evaluate the Growth and yield response of marigold to potting media containing different ratio of manures.The experiment was conducted in Randomized complete block Design (RCBD). The study comprised of five different potting media containing different ratio of manures treatments including T_1= Soil (S)–control, T_2 = 50% Farmyard manure + soil, T_3 = 50% poultry manure + soil, T_4 = 50% silt + soil, T_5 = 30% Farmyard manure + 30% poultry manure + soil, Seedlings of marigold were transplanted in Pots. each treatment was replicated three times.

Observation Recorded

- Plant height (cm),
- Number of branches per plant
- Number of flowers per plant

- Fresh biomass of flower (g)
- Flower diameter (cm)

Statistical Analysis
Data was statistically analysis to determine superiority of the treatment using ANOVA and least significant difference (LSD) tests. All statistical tests were performed using the computer software Statistic (Ver.8.1).

Procedure for Recording Observation
Plant Height (cm):
Three plants of each treatment were selected at random from experimental units and their height was measured from ground surface to the top with the foot scale and the average tallness was worked out in (cm) at the time of following.

Number of Branches Plant^{1}:
Average number of branches of three plants randomly selected from each treatment was counted visually and there by averages was done.

Number of Flowers Plant^{1}:
Average number of flowers of three plants randomly selected from each treatment was counted visually and there by averages was done.

Number of Leaves Plant^{1}:
Average number of leaves of three plants randomly selected from each treatment was counted visually and there by averages was done.

Fresh Biomass of Flower (g):
The fresh flower weight was recorded to plant-1 each replication of every treatment was measure by analytical balance.

RESULTS AND DISCUSSION

Plant Height (cm)
The marigold plant height results shown in figure 1 showed significant effects of various potting media. ANOVA showed that plant height (cm) was very significantly (P <0.05. The treated average of plant height (cm) was very significant. The result of maximum plant height (15.113 cm) is T_5= 30% Farmyard manure + 30% poultry manure + soil, followed by T_4= 50% silt + soil. Observed (14.997 cm). Plant height decreased in T_3= 50% poultry manure + soil, T_2= 50% Farmyard manure + soil, (13.033) and 10.703). As a further result, a minimum plant height (10.304 cm) of T_1= Soil =Control-No manure was observed.

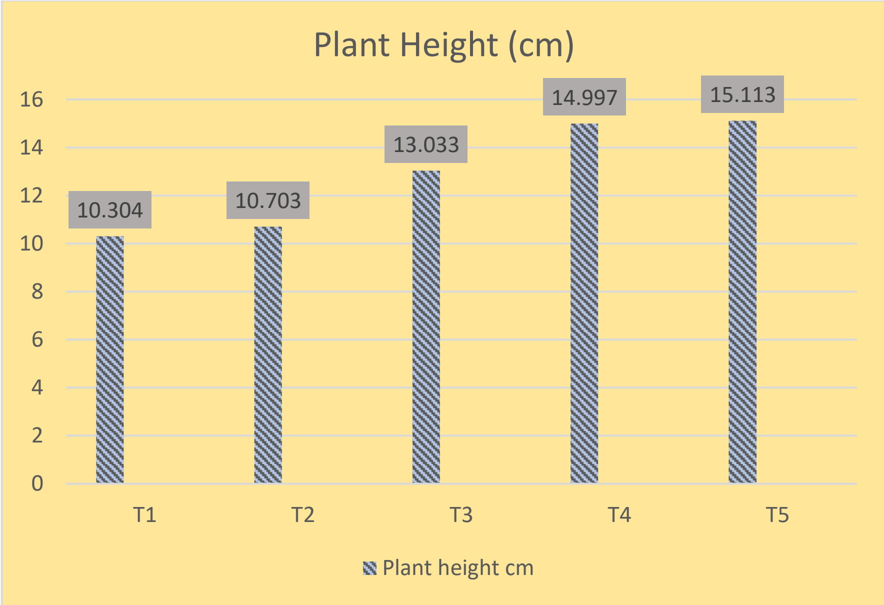

Figure No.1: Response of potting media on plant height (cm) of marigold.

Number of Branches per Plant

The branches per plant results shown in Table 1 showed significant effects of various potting media. T_5= 30% Farmyard manure + 30% poultry manure + soil resulted in more branches per plant (4.64), closely followed by T_4= 50% silt + soil and T_3= 50% poultry manure + soil, (4.54) and (3.90) branches per plant, respectively. The decreased in T_2= 50% Farmyard manure + soil caused a decline in branches to (3.15) per plant. And while T_1= Soil =Control-No manure further decreased branches to (2.88) per plant.

Table no 1: Response of potting media on number of branches per plant and Number of flowers per plant of marigold.

Treatment	No. of branches per plant	No. Of flower per plant
T_1 =Soil =Control-No manure	2.88	16.31
T_2 =50% Farmyard manure + soil	3.15	16.90
T_3 =50% poultry manure + soil	3.90	20.72
T_4 =50% silt + soil	4.54	23.94
T_5 = 0% Farmyard manure + 30% poultry manure + soil	4.65	24.13
Probability Value	0.0000	0.0001
Fisher Value	35.77	27.14
CV %	6.06	6.07

Number of Flower Plant

The marigold flowers per plant results shown in table 1 showed significant effects of various potting media. ANOVA showed that number of flowers was very significantly ($P < 0.05$) affected by various ratio of manures treatment. It is evident from the results that 30% Farmyard manure + 30% poultry manure + soil T_5 produced maximum Number of flowers per plant (24.13), followed by (23.94) and (20.72) flowers per plant recorded in treatments T_4= 50% silt + soil and T_3= 50% poultry manure + soil, respectively. The treatments based on 50% Farmyard manure + soil =T_2 resulted in (16.90) flowers per plant and minimum number of flowers per plant (16.31) was observed from T_1 = Soil =Control-No manure.

Fresh Biomass of Flower (g)

The Fresh biomass of flower (g) of marigold results presents in figure-2 showed important effect of various potting media. The mean of treatment for Fresh biomass of flower (g) was very significant. Results for maximum Fresh biomass of flower (210. .45 g) were observed at T_5= 30% Farmyard manure + 30% poultry manure + soil followed by T_4= 50% silt + soil (204.84 g). Fresh biomass of flower (g) decreases in T_3 = 50% poultry manure + soil, and in 50% Farmyard manure + soil =T_2 Fresh biomass of flower (g) (192.75, 174.52 g). Was obtained. As a further result, a minimum Fresh biomass of flower (150.26 g) from T_1 = Soil =Control-No manure was observed, ANOVA demonstrated that Fresh biomass of flower (g) treated with various pinching was very significant ($P < 0.05$).

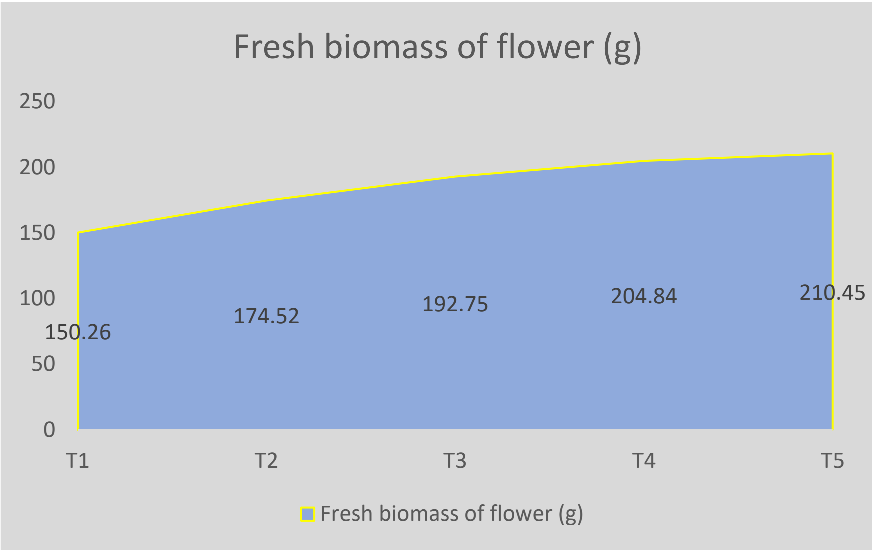

Figure No.2: Response of potting media on fresh biomass of flower (g) of marigold.

Flower Diameter (cm)

The flower diameter of marigold results shown in figure 3 showed significant effects of various potting media. 30% Farmyard manure + 30% poultry manure + soil = T_5 resulted in diameter of flower cm (15.11), closely followed by T_4= 50% silt + soil and T_3 = 50% poultry manure + soil with (14.99) and (13.03) diameter of flower cm, respectively. The decreased in T_2 =50% Farmyard manure + soil caused a decline in diameter to (10.71) of flower cm. while Soil =Control-No manure = T_1 further decreased flower diameter cm to (10.34)., ANOVA demonstrated that Diameter of flowers treated / growing in different ratio of manure was very significant ($P < 0.05$).

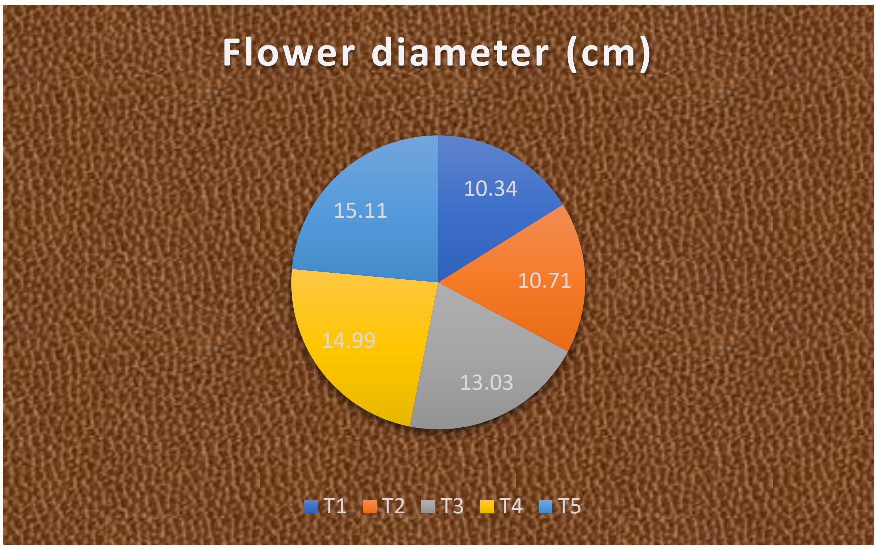

Figure No.3: Response of potting media on flower diameter (cm) of marigold.

Discussion

Marigold is a heavily feeder and generally has the longer blooming period (Aminifard, et al 2014). Increase in the Silt and Farmyard manure ratio increased total number of flower and inflorescence number (Litoriya, N. S., Gandhi, K. & Talati, J. G., (2014). The marigold receiving Canal silts + poultry manure + + farmyard manure (2:1)) resulted in (31.15 cm) plant height, (50.06) leaves plant-1, (53.83) days to flower bud initiation, (21.50) number of flowers per plant-, (11.58 g) weight of single flower, (33.51 mm) diameter of single flower and (57.00) days to flower persistence. After reviewing the data of this study, it was determined that marigold growth and flowering behaviors showed significant and positive response to farmyard manure ratios, and marigold treated with Canal silt + FYM (1:1) dust produced the longer plants with heavier and more flowers and blooming period as well. The response of varieties used in the study was also pronounced to different farmyard manure ration. The "Inca orange" produced much higher results than the "Bonanza harmony." (Koide, et al (1999) , Barman, D., Datta, M. De, L. C. & Banik, S. (2017). found the greater height of the plants with the application of FYM and poultry in marigold plants.

CONCLUSION

Result of this experiment show significant Growth and yield response of marigold to potting media containing different ratio of manures.has effect on the growth and yield of marigold, including Plant height (cm), number of branches per plant, number of flowers per plant, fresh biomass of flower (g), and flower diameter (cm).

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Field Performance Evaluation and Multiplex SNP Marker Application of Selected Varieties of Mungbean [*Vigna radiata* (L.) R.Wilczek]

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Abstract:
Mungbean [Vigna radiata (L.) R.Wilczek] is a drought tolerant orphan legume crop. The aim of the present study was to evaluate fifteen (15) varieties of mungbean using phenological data, yield attributes and SNP marker assisted tools. Field trial was established following standard agronomic practices. Molecular characterization was carried using a multiplex of SNP primers. Data analysis was done using Minitab 17.0 for data description, test of dependence, correlation and clustering options. Polymorphism Information Content (PIC) was calculated using standard the formula. Results confirmed a successful establishment and adaptability of mungbean varieties in the study area. Growth, flowering and yield attributes were excellent.RMG-344 was the best variety in phonological (flowering) and pod yield. Average grain yield was779 kg/ha where COGG-912 produced the highest grain yield of 995.31kg/ha followed by, MH-421 (984.33kg/ha) and ML-818 (933.57kg/ha). Plant biomass had the highest variability among all traits. SNP markers employed were highly polymorphic (average PIC was 0.849). A multiplex of CLM0115, CLM0118 and CLM0119 markers were the most polymorphic in terms of number of polymorphic bands produced, percentage polymorphism and PIC value. ML818 variety was the most divergent variety based on morphological attributes while molecular data showed five different groups of varieties that differ in their genetic constitution for an unknown trait that may be of interest to breeders. IC-39298 variety was ungrouped in the molecular data. The crop is suitable for adoption as a resilient, early maturing and high yielding legume crop. Selected varieties with quality agronomic traits such as flowering time, pod and seed yield should be included in breeding program. The highly polymorphic markers should be included in mungbean breeding work

Keywords: Mungbean, Evaluation, Performance, Markers.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) R.Wilczek], a member of the family Fabacea, is a drought tolerant legume also known as the green gram. It was domesticated in Persia (Iran) where its progenitor *Vigna radiata* subspecies *sublobata* occurred wild (Belay *et al.*, 2019). Cultivated mungbean later spread to China and Southeast Asia. Today, it is mainly cultivated as a food crop and major ingredients of different food products in East Asia, Southeast Asia and India as an annual crop (Zhu *et al.*, 2018; Baraki *et al.*, 2020). India is the largest producer of mungbean which accounts for 54% of world production (Baraki *et al.*, 2020). Farmers in some African countries notably Kenya, Tanzania, Ethiopia, Mozambique and Uganda have keyed into the production of mungbean as a staple protein source (Nair *et al.,* 2019; Baraki *et al.,* 2020). It is cultivated to supplement the protein needs in seasons of insufficient rainfall. Apart from drought, studies have shown high tolerance of mungbean varieties to other stress conditions such as salinity (Sehrawat and Jaiwal, 2014; HanumanthaRao *et al.,* 2016).

Productivity of mungbean is reportedly higher than many legume crops (Sehrawat and Jaiwal, 2014; Kumar *et al.,* 2017; Sehgal *et al.,* 2018). The seeds are loaded with quality nutrients sufficient enough to address the challenges of malnutrition. High adaptability to different environments, low input costs, and high yield make it highly suitable for cultivation in countries with protein deficiency (Baraki *et al.,* 2020). The earlier introduction of some mungbean varieties into the humid and dry agro-ecosystems of Nigeria have failed to yield success due to lack of robust evaluations of a broad germplasm needed for varietal selections and adoption by farmers (Asari *et al*., 2019). Mungbean was among the legumes evaluated for potential use in dryland farming systems as a drought-tolerant crop that can provide a resilient response to the changing climate. From the previous evaluations done so far (2019-2021), some varieties were selected based on their potential agronomic and breeding values of interests to both the researchers and the growers.

The present study became necessary at this crucial moment when food security is the target of the United Nations in the present decade. Hunger eradication in African countries is possible when integrated approaches are adopted. Part of these strategies is to seek alternative legume crops that that are highly stress tolerant, disease resistant and high yielding that can be produced at minimal costs. There is need to evaluate mungbean in terms of adaptability for possible adoption in African countries. Previous projects established at the Joseph Sarwuan University Makurdi has resulted in the identification and selection of some varieties with excellent breeding qualities. In the current work, the phenotypic and genotypic studies are necessary to further characterize these selections using molecular markers and identify those that can serve as templates in future breeding work for possible release of quality seeds that can be adopted by the Nigerian farmers. The aim of the present study was to evaluate fifteen (15) selected varieties of mungbean using phenological, yield attributes and marker assisted tools.

MATERIALS AND METHODS

Study Site

The experimental site was an expansive land along Gbajimba Road allocated to the College of Agronomy, JOSTUM for legume trials, Makurdi town is the headquarter of Makurdi LGA. (Latitude 7^{o}38'N-7^{o}50'N; longitude 8^{o}24'E-8^{o}38'E) with a landmass of about 16 kilometres (km) in radius and a population of 300,377 (Abah, 2013). The rainy season lasts from April to October with 5 months of dry season. The mean annual temperature of the area is 1173mm but the temperature is high throughout the year ranging between 22.5^{o}C and 40^{o}C. The vegetation is of the Guinea Savannah type (Abah, 2013). Benue State is popularly known as the food basket of the nation. Agricultural activities are high in the study area especially among the rural dwellers who cultivate diverse types of fruit and vegetable crops, pulses and cereals at both subsistence and commercial level.

Planting Season

The study was carried out in the 2022 planting season. Field was established on 21st August, 2022 alongside other legume trials evaluated by the Department of Plant Breeding, College of Agronomy, Joseph Sarwuan Tarka Makurdi, Benue State, Nigeria (JOSTUM).

Seed Source and Management

Fifteen (15) varieties of mungbean seeds were sourced from the germplasm of the Molecular Biology Laboratory, Department of Plant Breeding, College of Agronomy, JOSTUM. These varieties selected from previous trials for continuous evaluations based on the unique agronomic values of interest to the breeders. Seeds were packaged in sealed envelopes labeled with names of each variety written on them. The varieties are: GAM-5; Ganga-8; MH-421; SML-668; RMG-344; RMG-492; COGG-912; ML818; MUM-2; IC-39298; IC-39300; IC-39368; IC-39375; IC-39500 and IC103245

Field Preparation, Experimental Design and Planting

Land clearing, ploughing and ridging were done using mechanized farming system established by the STOL project team. A total of 15 varieties were planted in a RCBD (Randomized Complete Block Design) of 3 blocks and 3 replications to determine varietal performances. Four (4) seeds of each variety were sown in 4 rows of 4 m length spaced 0.60 m apart and within -row plant to plant distance of 0.15 m to give a gross plot size of 4 x 3 m (12 m^2) (IBPGR/IPGRI, 2019). A mixture of pendilin and gramoxone at a rate of 2.5 L ha^{-1} gramoxone and 1 L ha^{-1} pendilin was applied immediately after planting using Knapsack sprayer. Hoe weeding was used just before flowering to remove subsequent weeds. Fertilizer rate 15:15:15 was applied using a compound fertilizer (NPK 15; 15; 15) to provide 15 kg each of N, P, and K ha^{-1} as starter dose.

Varietal Characterization

Phonological and yield related data were collected using the IBPGR/IPGRI descriptors of 2019. Data entry was done in the field log book aided by the use of visual observation, meter rule, portable digital weighing balance, pencil, erasers and pen. The following morphological traits were characterized in the field: number of primary branches, days to flowering, days to 50% flowering, number of pods per plant, days to maturity, pod length (cm), pod weight (g), seed weight per plot (g), 1000 seed weight (g)number of seeds/pods, average biomass (kg) and yield (kg/ha)

Molecular Characterization

DNA extraction was done using the CTAB method (Omoigui *et al.*, 2015). A suitable protocol was established after the optimization stage. A total of ten (10) legume-specific SNP primers was used for DNA amplification stage as listed in *Table 1*. A Multiplex PCR (Polymerase chain reaction) was performed on a heated lid thermal cycle using the thermal cycle operated as follows: 35 cycles of denaturation at 94 °C for 30s, followed by annealing temperature at 57.5 °C for 30s and extension at 72 °C for 2 min. A final extension cycle of 10 min at 72 °C was added to ensure completion of the final amplification products as described by Omoigui *et al*. (2015). Amplicons were resolved in an agarose-based gel electrophoresis (0.8% agarose powder in 1xTAE buffer; 5uL of ethidium bromide) Galileo Bioscience tank connected to Consort EV243 electrophoresis power supply. The gel ran at 120v for 45 minutes. DNA purity and quality was checked using UV spectrometer light (Omoigui *et al.,* 2015). The ethidium bromide-stained gel was visualized on an UV transilluminator and photographed using a digital camera

Table 1: SNP Primers and their sequence

Primer	Forward sequence (3-5’)	Reverse sequence (5-3’)
CLM0110	CAGGCTGAAAGTGCCATTAT	GGAAGTTTCACCACCCTTTC
CLM0111	ATAGGAATTGGACTGGGCTT	TGCAAAGGGTTGATTTGAA
CLM0112	GCTGCCATGTCCATTAGAGT	GAGAGGACTTGGGTTCGAGT
CLM0113	TCAAGAATCTTTAGACCGAA	CCTGAGTTTAATTATTTGTTTCA
CLM0114	TTCCTTAGCCAAAGTGTTCC	TCAACGACAGCGTTATCAAA
CLM0115	TTTCCATTGCATTTATTCCAC	TCAGGAGACAGAATGGAAGG
CLM0116	GACTGCTACATGGCCTCAAA	CCTGCATTTACACATTGTCTC
CLM0117	AAGACAAGCTCCCTGGAAGT	GTCATTATGCATTGGGCATT
CLM0118	AAATTCTGCAACGACTACGC	TGCATTCTTCCGTAGTGTGA
CLM0119	GAGATGTTGAGATGGTGGCT	CCTTGGTCATTGAACCTCTC

Scoring of Gel Images and Analysis

Only clear and unambiguous bands were scored. Markers were scored for the presence and absence of the corresponding band among the genotypes. The scores ‘1’ and ‘0’ indicate the presence and absence of bands, respectively to generate a binary matrix. Data analysis was done using the Microsoft Excel Workbook and Minitab 17.0. Statistical operations carried out on field data were: data description with graphical summaries for each trait, test of dependence using the Chi-square tool, correlation analysis using the Pearson Product Moment method. Polymorphic bands were analyzed for each primer used. Polymorphism Information Content (PIC) was calculated using the formula adopted by Weir (1990) and Xu (2010).

$$PIC = 1 - \Sigma\, pi2$$

Where *pi* is the frequency of the *i* th allele for each SSR marker *i* th summed across all alleles for the loci. Cluster analysis was performed on morphological and molecular data each resulting in the construction of dendrogram using the Average Linkage method measured on Euclidean Distance.

RESULTS

Table 2 gives a description of mungbean plants in the field. The varieties produced between 0.6 and 2.6 branches in the field. They flowered from day 25 to 32 after planting while the mean flowering day was 29.2±1.86. Varieties produced 50% flowering within 32-36 days. Mean number of pods produced per plant was 36.78±12.0 ranging from 17 to 55.8 pods among the plants. The 1000 seed weight varied between 36.5g and 49.3g. Seed weight per plot measured between 387.50g and 658.30g. Total yield grain varied from 504.30kg/ha to 995.30kg/ha among the varieties while average yield produced was 778.5±160.2 kg/ha**.** Plant biomass had the highest coefficient of variability (45.7%) followed by the number of primary branches (32.75%), number of pods per plant (32.6%), pod weight (20.8%) and yield (20.6%). Distribution of grain yield was not normal (A-squared = 0.54, p>0.05) using the Anderson-Darling normality test (skewness= -0.36) while 95% confidence interval for the mean was between 646.9kg/ha to 867.2kg/ha (*Figure 1*). Top three high yielding varieties were COGG-912 (995.31kg/ha), MH-421 (984.33kg/ha) and ML-818 (933.57kg/ha) (*Figure 2*). The overall yield measurement in kg/ha was dependent on the type of varieties (χ^2 =461.30, P<0.05). Chi- square distribution showed that the observed values in yield were higher than the expected values in MH-421, COGG-912, ML-818, RMG-344, SML-688, RMG-492, MUM-2, IC-39298 and IC-39300 (*Figure 3*). ***Table 3*** gives the correlation coefficients of the quantitative characters in a matrix table. Moderate positive correlation (r= +0.40-0.69) exist between: pod weight and number of pods per plant (r=0.574); number of seed per pod and number of pods per plant (0.521); seed weight per pot and pod weight (r=0.651), biomass and number of seed per pod (r=0.673). Moderate negative correlation was found between pod length and day to maturity (r= -0.551). Yield in kg/ha positively correlated with both pod weigh (r= 0.790) and seed weight per plant (r= 0.701). Cluster analysis done using morphological data showed that similarity coefficient level was between 73.3 and 93.7 resulting in a distance of between 168.9 and 39.8 respectively among the varieties. The resulting dendrogram of pehenotypic data constructed using the single linkage method (*Figure* 4) gave 3 clusters where varieties displayed some levels of similarity in phonological and yield attributes. The most related varieties were RMG-492 and IC-39300. Others such MUM-2 and IC-39298 were both similar but were separated from other varieties thus forming the third cluster. The most divergent variety was the ML818 (similarity coefficient = 76) being the highest in pod weight (937.55g), seed weight per plot (658.32g). It had a very high pod yield (54.1) and the second highest producer of total grain yield (933.57kg/ha).

Phenotypic assessment has revealed a successful crop establishment as shown from the luxuriantly growth, appealing vigour and good performances of the varieties although with some levels of variability. The above observation could be an indication of high ecological adaptation and tolerance to the biotic and abiotic environment including the climatic and edaphic related factors as suggested in other studies (Yashvir and Rex, 2018; Nair *et al.,* 2019). This report is also in tandem with other findings that mungbean is a resilient crop that tolerates different environment (Yashvir and Rex, 2018; Nair *et al.,* 2019). There appears to be a strong indication of high influence of the environment on phonological and yield related traits of the fifteen mungbean varieties in the field. The impact of main effects of environment (E) and the interactions on yield of genotypes (G) was earlier established (Yashvir and Rex, 201) some of which were related to physiological traits including time to flowering and maturity. Average flowering time was 29 days while maturity time was 75 days. Time to flowering in mungbean was subject to both genetic and environmental control (Miko, 2018). The photo-thermal control on flowering time has been extensively modeled (Kaur *et al.,* 2015; Sharma *et al.,* 2016; Fathy *et al.,* 2018; Nair *et al.,* 2019; Baraki *et al.,* 2020). The reported flowering time of mungbean appeared similar to cowpea, an indication that mungbean completes its lifecycle quickly. Early flowering is a good characteristic as harvesting could be achieved before the severity of drought in semi-arid regions. This agrees with the findings of Kaur *et al*. (2015) who studied the inheritance of time to first flowering in photo-insensitive mungbean. According to the authors, early maturity as determined by early flowering is an important agronomic trait that is crucial in the adaptation of annual crops. In the present work, RMG-344, RMG-492 and IC-393638 have been selected in this study as the best mungbean varieties with earliest flowering time. Pod characteristics in terms of number of pods per plant, pod sizes, pod weight and number of seeds present in each pod of the evaluated mungbean varieties are excellent attributes that may attract the attention of growers. RMG-344 (the best variety in number of pods) produced as high as 46 pods per plant and closely followed by SML-668 that produced 44 pods per plant. These two varieties have been selected for inclusion in breeding work. However, number of these two varieties was the best in terms of number of seeds per pod. SML-668 and MUM-2 were selected for this trait.

The average grain yield of 779 kg/ha established in this work was higher than 750kg/ha reported by Olson *et al.* (2011) but lower than the values reported by Belay *et al* (2019) and Baraki *et al.* (2020) who worked on mungbean. Due to several challenges, the average cowpea production in West Africa was reported to be as low as 358kh/ha (FAO, 2000), although some improved cowpea varieties had slightly higher yield with improved resistance (Omoigui *et al.,* 2017). Thus, farmers can take advantage of high yield attributes of mungbean as an excellent plant-based protein source to supplement cowpea production. Top high yielding varieties including COGG-912 (995.31kg/ha), MH-421 (984.33kg/ha) and ML-818 (933.57kg/ha) should be considered in breeding program to improve other low yielding varieties. Baraki *et al.* (2020) who worked on the genotype x environment interaction and yield stability analysis of mungbean genotypes reported a significant variation in grain yield among the genotypes, environment and genotype by environment interaction. In the present study, it was established that yield is positively influenced by pod and seed weight. Plant biomass had the highest variability among the evaluated varieties and it was moderately influenced by number of seed per pod. As a weight related parameter, plant biomass has been extensively reported to be affected by the amount of water present in a living system (Sharma *et al.*, 2016; Yashvir and Rex, 2018; Baraki *et al.*, 2020). It may be inferred that the fifteen varieties differ in the amount of water present as a function of water absorption and utilization. It should be noted that mungbean is a drought tolerant legume crop. In other studies, biomass (dry matter) was the most important yield determinant of with over 90% of the total variation in yield being accounted by this trait (Sharma *et al.*, 2016; Fathy *et al.*, 2018). Developing mungbean plants with a root system that is able to draw more moisture from the soil could help reduce this extreme phenological and morphological sensitivity to water stress situations (Baraki *et al.*, 2020).

Table 2: Description of quantitative characters

Traits	Mean±SD	CV%	Min	Max
Number of primary branches	1.81±0.59	32.68	0.60	2.60
Days to First flowering	29.2±1.86	6.37	25.00	32.00
Days to 50% Flowering	34.93±1.16	3.33	32.00	36.00
Number of pods per plant	36.78±12.00	32.62	17.60	55.80
Days to Maturity	75.27±1.10	1.46	73.00	76.00
Pod length (cm)	8.05±0.33	4.06	7.60	8.80
Pod weight (g)	693.7±144.5	20.83	444.40	937.50
Seed weight per plot (g)	495.7±88.4	17.83	387.50	658.30
1000 seed weight (g)	40.39±3.22	7.96	36.50	49.30
Number of seeds per pod	10.75±0.82	7.65	9.50	12.50
Average Biomass (kg)	0.43±0.20	45.74	0.15	0.94
Yield kg/ha	778.5±160.2	20.57	504.30	995.30

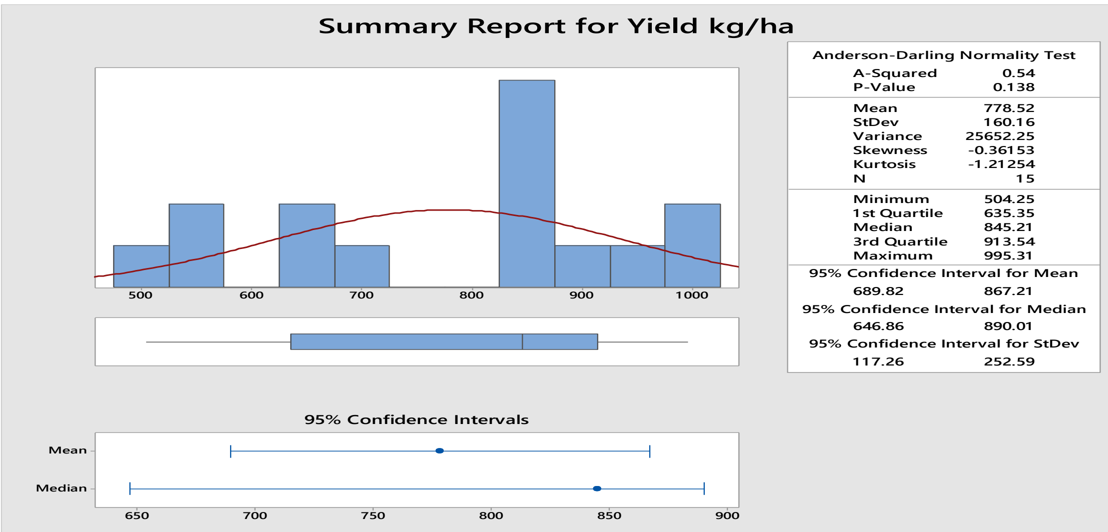


Figure 1: Graphical summary for Yield (kg/ha)

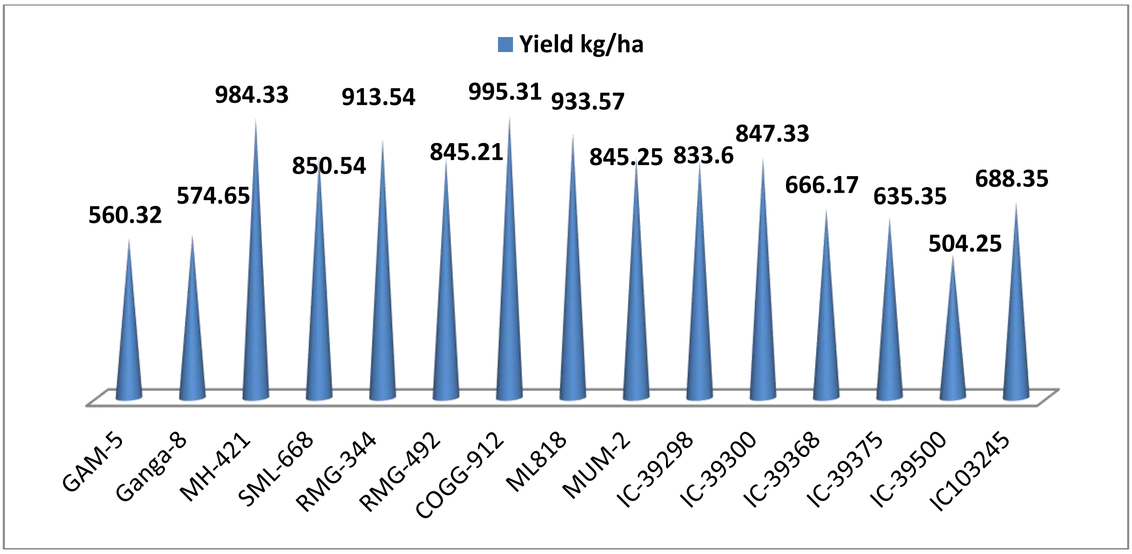
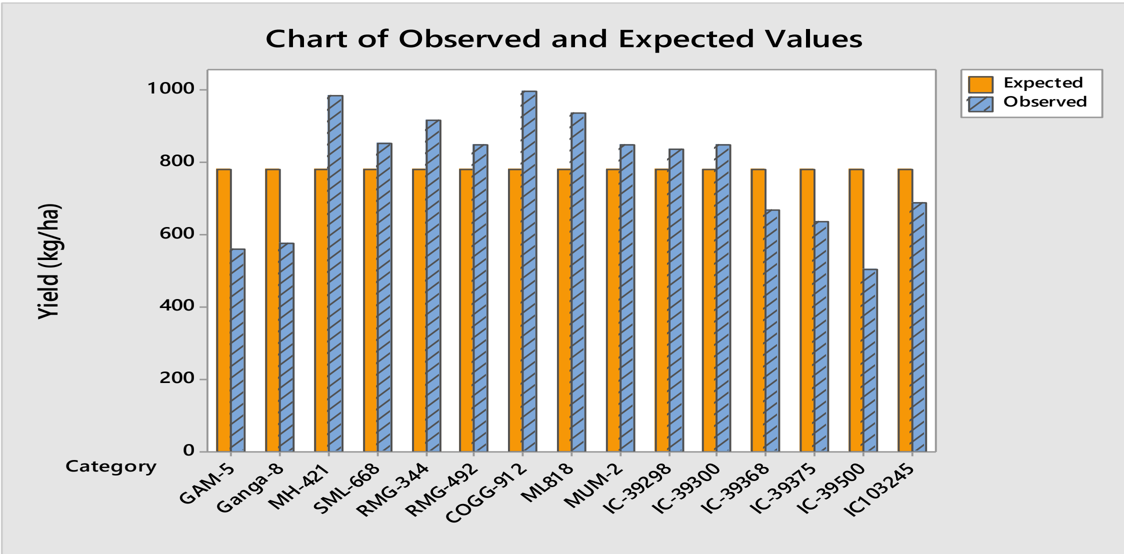


Figure 2: Performances in grain yield among mungbean varieties



χ^2 (Yield) = 461.302, P=0.000 (P<0.05)

Figure 3: Test of dependency between seed weight and mungbean varieties

Table 3: Pearson Correlation Matrix of Quantitative Traits

Traits	NPB	DFF	D50F	NPP	DM	PL	PW	SWP	1000SW	NSP	AB
DFF	-0.016										
D50F	-0.258	**0.667**									
NPP	0.330	0.188	-0.168								
DM	0.027	-0.028	0.127	-0.368							
PL	0.056	0.371	0.046	0.361	**-0.514**						
PW	-0.190	-0.246	-0.235	**0.574**	-0.290	0.097					
SWP	-0.321	-0.174	-0.311	0.464	-0.210	0.242	**0.651**				
1000SW	0.024	0.107	0.072	-0.335	0.023	0.436	-0.479	-0.192			
NSP	0.214	0.288	0.220	**0.521**	-0.339	0.199	0.120	-0.094	-0.108		
AB	0.188	0.052	0.069	0.344	-0.499	0.202	0.460	-0.075	-0.181	**0.673**	
Yd	-0.341	-0.221	-0.296	0.488	-0.350	0.194	**0.790**	**0.701**	-0.377	0.162	0.337

Number of primary branches =**NPB**; Days to First flowering=**DFF**; Days to 50% Flowering=**D50F**; Number of pods per plant= **NPP**; Days to Maturity= **DM**; Pod length (cm)= **PL**; Pod weight (g)= **PW**; Seed weight per plot (g)= **SWP**; 1000 seed weight (g)= **1000SW**; Number of seeds per pod= **NSP**; Average Biomass (kg)= **AB**; Yield=**Yd**

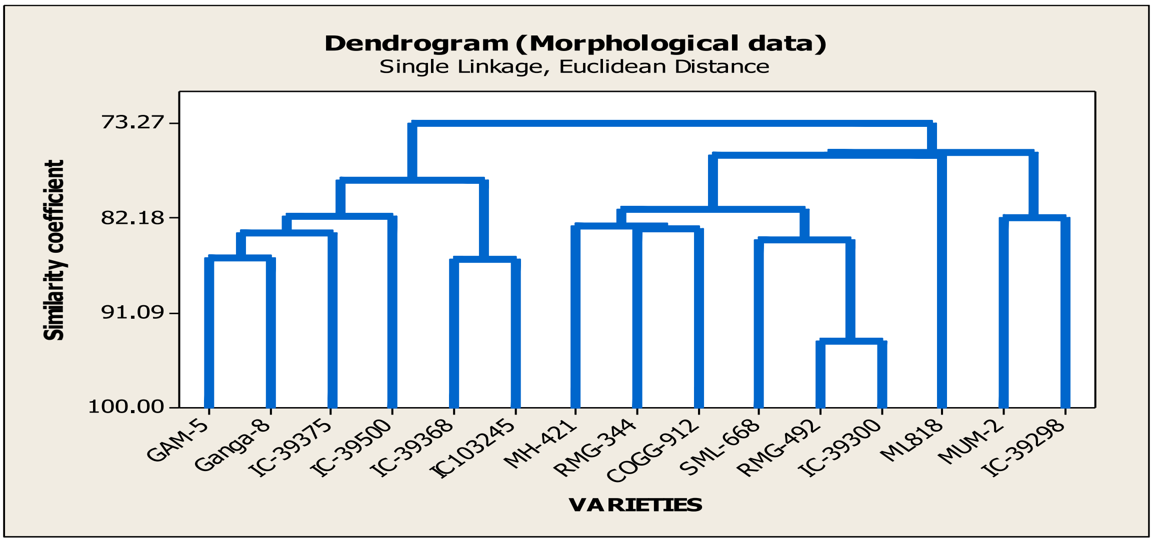


Figure 4: Dendrogram showing clustering pattern of mungbean accessions using morphological data

All the varieties possessed a specific trait (500bp) but different heritability pattern as some varieties showed double bands while others showed single bands at the same locus (*Plate 1*). CLM0110, CLM0112 and CLM0113 multiplex primers produced double bands in all except in three varieties resulting in 5 polymorphic bands where % Polymorphism was 33.3% and Polymorphic information content (PIC) was 0.845. CLM0114 and CLM0116 multiplex primers produced double bands in 8 out of 15 varieties resulting in 4 polymorphic bands where % Polymorphism was 26.7% and PIC was 0.779. CLM0115, CLM0118 and CLM0119 multiplex primers produced 6 double bands resulting in 6 polymorphic bands where % Polymorphism was 40.0% and PIC was 0.922, thus the most polymorphic primer set. All primers gave an average of 5 polymorphic bands and 0.849 PIC (*Table 5*). Similarity coefficient among the varieties ranged from 22.06 to 100.0 while genetic distance was between 1.35 and 0.00. Intermediate similarity level (42.3) was observed. Average distance from centroid was 0.801while the maximum distance from centroid was 1.069. The resulting dendrogram (*Figure 5*) gave 2 clusters and 5 groups of varieties that are similar in banding pattern (5 polymorphic groups). Groups containing two clustered varieties were GAM-5 and IC-39375 as well as IC-39368 and IC-103245. Other groups comprised 3-4 varieties. The only ungrouped variety among them was IC-39298.

The use of SNP (single nucleotide polymorphism) in this study has proven to be a good marker candidate in showcasing the relatedness and divergence among the mungbean varieties. As the name implies, each SNP reveals the polymorphism that exists among varieties differing by a single nucleotide at a particular locus. The primer combinations were polymorphic with high PIC values. According to Nelson *et al*. (2016), PIC values of 0.5, 0.4 and 0.2 are classified as highly informative, moderately informative and little informative respectively. The average PIC of 0.849 obtained in this study was higher than values obtained in many studies (Dhaliwal *et al.*, 2014; Kumar *et al.*, 2016). A marker is said to be polymorphic if it has at least two alleles (Mason, 2015). Here, we reported a multiplex of CLM0115, CLM0118 and CLM0119 as the most polymorphic in terms of number of polymorphic bands produced, percentage polymorphism and PIC value. These markers may be useful in future breeding work of mungbean. This outcome is consistent with the report given by Omoigui *et al.* (2015) who determined the suitability and use of two molecular markers to track race-specific resistance to *Striga gesnerioides* in cowpea. Information obtained from cluster analysis reveals ML818 as the most divergent based on morphological attributes while molecular data showed five different groups of varieties that differ in their genetic constitution for an unknown trait that may be of interest to breeders. IC-39298 variety should be investigated further to ascertain why it was unrelated genetically.

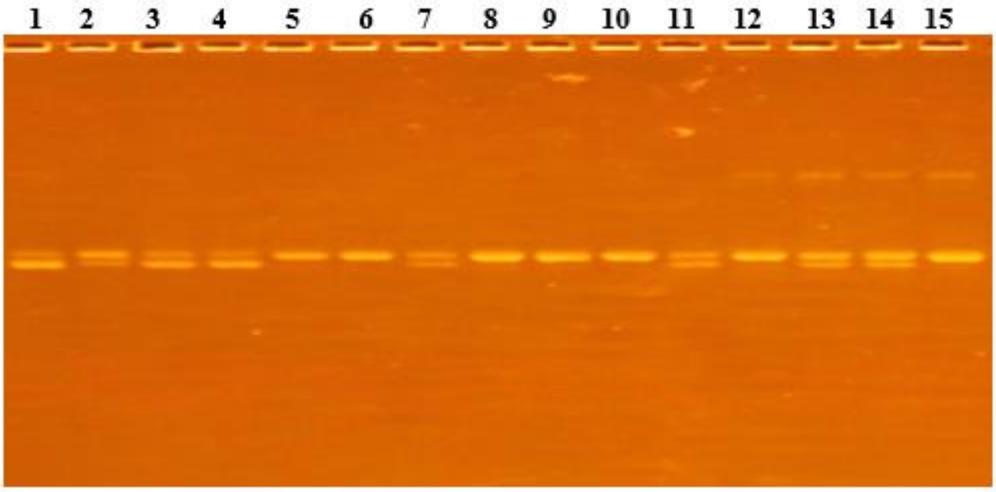


Plate 1: DNA band profile of CLM0114 and CLM0116 Multiplex Primers

Table 5: Multiplex Primers and their Polymorphism Information Content (PIC)

Primer name	Forward Sequence	∑Polymorphic bands	% Polymorphism	PIC 1 - Σ *pi*2
CLM0110	CAGGCTGAAAGTGCCATTAT	5	33.33%	0.845
CLM0112	GCTGCCATGTCCATTAGAGT			
CLM0113	TCAAGAATCTTTAGACCGAA			
CLM0114	TTCCTTAGCCAAAGTGTTCC	4	26.67	0.779
CLM0116	GACTGCTACATGGCCTCAAA			
CLM0115	TTTCCATTGCATTTATTCCAC	6	40.00%	0.922
CLM0118	AAATTCTGCAACGACTACGC			
CLM0119	GAGATGTTGAGATGGTGGCT			
Total		15		2.546
Mean		5.0		0.849

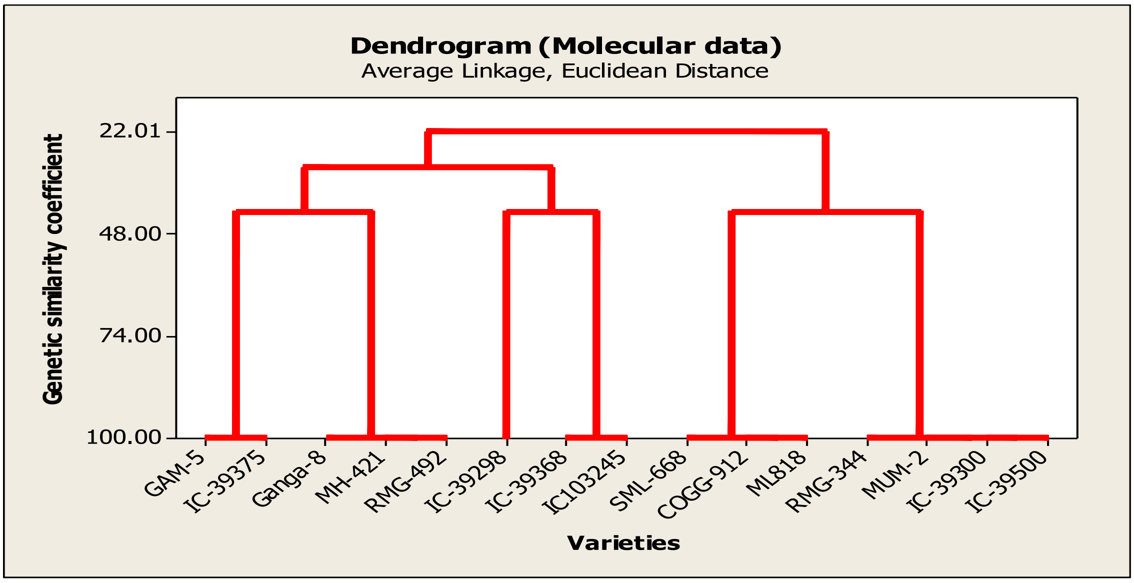


Figure 5: Dendrogram showing clustering pattern of mungbean accessions using molecular data

CONCLUSION

This study confirmed a successful establishment and adaptability of mungbean varieties in the study area. RMG-344 was the best variety in phonological (flowering) and pod yield. Average grain yield was779 kg/ha where COGG-912 produced the highest grain yield of 995.31kg/ha). ML818 variety was the most divergent variety based on morphological attributes while molecular data showed five different groups of varieties that differ in their genetic constitution. The crop is therefore suitable for adoption and cultivation among Nigerian farmers being a resilient, early maturing and high yielding legume crop. Selected varieties with quality agronomic traits such as flowering time, pod and seed yield should be included in breeding program. The highly polymorphic markers selected (CLM0115, CLM0118 and CLM0119) should be included in mungbean breeding work.

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Proximate and Anti-Nutritional Analyses of Okra Seeds (Silver Queen, Star of David, and Clemson Spineless)

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Abstract:
This study evaluated the nutritional qualities in seeds of three varieties of okra (Silver Queen, Star of David, and Clemson Spineless) that are commonly cultivated in Makurdi, North Central Nigeria. Grand mean nutritional composition in okra fruits showed that carbohydrate was the highest class of food (43.7%) followed by protein (22.7%) and moisture (10.3%), fiber (9.2%), ash (7.2%) and lipid (6.9%). Result indicated that Silver Queen variety had the highest protein (22.93%), carbohydrate (45.57%), fiber (11.1%) and moisture (8.3%) while Clemson Spineless had the highest ash (7.07%) and lipid content (7.03%), although varietal differences are insignificant (P>0.05). Oxalate (0.51-0.88mg/100g) and cyanide (0.09-1.28mg/100g) were present as anti-nutrients but in very low concentration with insignificant varietal differences (P>0.05). Phytic acid content was the highest anti-nutrient quantified and it differed significantly among the varieties (P<0.05). Phytic acid was 49 times higher than oxalate and 59 times higher than cyanide in okra seeds tested. Phytic acid was lowest in Silver Queen (46.7mg/100g) while the highest amount was found in Clemson spineless (64.8mg/100g). Pearson's correlation showed negative coefficients between phytic acid and all nutrients except carbohydrate (0.840). From these findings, it could be deduced that Silver Queen variety was the best variety of nutritional values being high in carbohydrate, protein, fiber and moisture together with minimal level of anti-nutrients. Breeding efforts are needed to improve the nutrition of okra and reduce the anti-nutrients. This information is important to consumers in the quest to attaining national food security.

Keywords: Okra, Nutrition, Anti-nutrition, Breeding, Food security

INTRODUCTION

Okra fruit (*Abelmoschus esculentus* (L) Moench) popularly known as lady's finger is of the family Malvacea. It is native to Africa and therefore a popular vegetable crop in the tropical and subtropical regions cultivated in about 277,000 hectares of land in Nigeria (Ekwu and Nwokwu, 2013). Okra is one of the best soup condiments known so far and it is consumed in almost every home in Nigeria due to the ease of preparation and cost effectiveness. It contains quality nutritional properties such as water, protein, fat carbohydrate, fibre, ash and oil (Akinfasoye and Nwanguma, 2015). The fruit is a rich source of vitamin while the slimy mucilage has been reported to possess high carbohydrate contents and other medicinal values (Sale *et al.*, 2015). The crop was described as a multipurpose vegetable fruit crop (Dandena, 2012; Fekadu *et al.*, 2015).

The fruit has the potentials to help achieve food security in the vegetable segment due its low cost, ease of cultivation and preparation (Sanni *et al.*, 2015; Fagwalawa and Yahaya, 2016). However, it is under-utilized in many parts of Nigeria. Nutritional studies done so far focused on the fruits alone while the seed contents that form a bulky part is neglected (Habtamu *et al.*, 2014; Sale *et al.*, 2015) There are sketchy pieces of information on the nutritional values of the okra seeds. It is also uncertain if the okra varieties are devoid of toxic anti-nutrients that pose dangers to human health (Sale *et al.*, 2015; Arapitsas, 2018). Therefore, there is need to investigate three popular okra varieties (Silver Queen, Star of David and Clemson Spineless) sold in Makudi markets, Benue State, for their comparative nutritional values. The aim of the study was to investigate the proximate composition and determine the quantity of three anti-nutrients (cyanide, phytic acid and oxalate) in okra seeds. The specific objectives were to: determine the percentage composition of carbohydrate, protein, lipid, fiber, ash, moisture, oxalate, phytic acid and cyanide present in the three okra varieties; and also determine the relationship among the nutrients and anti-nutrients.

MATERIALS AND METHODS

The Study Area

This study was conducted in the General Biology Laboratory, College of Biological Sciences, Joseph Sarwuan Tarkaa University Makurdi, Benue State.

Sample Collection, Identification and Preparation

Seeds were sourced from the seed bank germplasm of the Botany Department of the same institution and authenticated by Dr. J.O. Olasan as Clemson spineless, Star of David and Silver Queen. Exactly 500g of each sample, labeled as V1-V3, was pounded into powder form and tied separately and stored till further use

Proximate Analysis

Moisture Content:

Moisture content was determined using the oven dry method (Adetuyi *et al.*, 2011). It was calculated as: by:

$$\% \text{ Moisture} = \frac{w2-w3}{w2-w1}\ x \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where, w1= weight of empty moisture can (petri dish); w2= weight of can and sample before drying; w3= weight of can and sample after drying

Crude Protein:

The micro-kjeldahl titration method was used where samples were mixed with 10ml of concentrated tetraoxosulphate (vi) acid in a kjeldahl digestion flask (Simone, 2014). The total nitrogen was calculated and multiplied by a factor 6.25 to obtain the crude protein content as % Crude protein =%N6.25.

$$\% \text{ N2} = \frac{(100\text{x})\text{Nx14xVfxT}}{\text{w x 100 x VA}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

W= weight of the sample; N = Normality of filtrate ((H_2So_4) = 0.02N; V_F = Total volume of the digest = 100ml; V_A = Volume of the digest distilled

Determination of Fat:

Fat content was determined by the solvent extraction method using a soxlet apparatus (Adetuyi *et al.*, 2011). The weight of the fat (oil) extracted was expressed as percentage of the sample weight.

$$\% \text{ of fat} = \frac{w2-w1}{w1} x \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

W = weight of the sample; W_1 weight of empty extraction flask

Ash Content Determination:
Ash cntent was determined using the furnace incineration gravimetric method (Adetuyi *et al.*, 2011). Percentage ash content was determined as

$$\% \text{ Ash} = \frac{w2 - w3}{w2 - w1} x \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

W_1 = weight of the crucible; W_2 = weight of sample in crucible; W_3 = weight of crucible + ash

Determination of Crude Fiber:
This was determined by the Weende method **(**Adetuyi *et al.*, 2011**)** and calculated as

$$\% \text{ Crude fibre} = \frac{\text{loss in weight incinertion}}{\text{weight of sample}} x \frac{100}{1} = \frac{w2 - w3}{\text{weight of sample}} \ldots\ldots\ldots\ldots (5)$$

W_2 = weight of crucible sample after washing and drying in oven; W_3 = weight of crucible + sample ash

Determination of Carbohydrates:
Carbohydrates was determined by Nitrogen free extraction (NFE) method (Adetuyi *et al.*, 2011). The NFE was calculated as

% NFE = 100 - % (a+b+c+d+e) .... (6)

Where: a= protein; b= fat; c= fibre; d= ash; e= moisture

Determination of Anti-nutritional Factors in Okra Seed:
Concentration of all anti-nutrients in okra seed was determined in mg/100g using standard methods (Habtamu *et a.*, 2014). The oxalate content was calculated by taking 1ML of 0.05M $KMnO_4$ as equivalent to 2.2mg oxalate. Phytic phosphorus (Pp) was determined and the phytic acid content was calculated by multiplying the value of Pp by 3.55 where Fe equivalent=1.15x titer value and Pp= titer valuex1.19x1.95. Therefore, phytic acid =1.95x1.19x3.55x titer value. Alkaline picrate colour development was used in the determination of cyanide where absorbance was read at 450nm against blank.

Data Analysis
Data was analyzed on the Minitab 17.0 software using the following statistical tools: Chi Square test of dependency; One way ANOVA ($P \leq 0.05$) and Pearson's correlation.

RESULTS AND DICUSSION

Table 1 shows the proximate composition of three varieties of okra: Silver Queen, Star of David, and Clemson Spineless. Silver Queen had the highest protein (22.93%), carbohydrate (45.57%), fiber (11.1%) and moisture (8.3%) while Clemson Spineless had the highest ash (7.07%) and lipid content (7.03%) among the three varieties. The observed differences in a particular nutrient for all varieties are insignificant statistically ($P>0.05$), Grand mean nutritional composition in okra fruits showed that carbohydrate was the highest class of food (43.7%) followed by protein (22.7%). Others are moisture (10.3%), fiber (9.2%) and ash (7.2%) while lipid content was the lowest (6.9%) in the fruit (figure 1).

The nutritional compositions in three okra varieties reported in this work are in tandem with the available data in literature (Adetuyi *et al.,* 2011; Olujobi and Ayodele, 2013; Habtamu *et al.,* 2014; Arapitsas, 2018), thus confirming its dietary values. In this work, only the seed component was investigated for proximate and anti-nutrients as only dry seeds were available in preserved forms with known identities. This study has further supported claims that the seeds are of valuable nutritional qualities in okra fruits and that consumers should avoid discarding them during food preparation. The reported amount of carbohydrate of 44.7% being the highest followed by protein (22.7%), moisture (10.3%), fiber (9.2%), ash (7.2%) and lipid (6.9%) has shown that the seeds are much more valuable than the entire okra fruits based on previous nutritional analysis done on the fruits by some researchers (Khomsug *et al.,* 2010; Roy *et al.,* 2014; Habtamu *et al.,* 2014). It thus suggests that the okra seeds present a form of balanced nutritional diet that should be embraced to reduce the level of malnutrition in developing countries. The seeds may therefore be incorporated into the production of industrial feeds and food supplements for humans and livestock. The three varieties studied have therefore showcased quality nutritional values. However, the Silver Queen variety seems to be the best.

Table 2 presents the anti-nutritional factors (cyanide, oxalate, and phytic acid) content in three varieties of cowpea. Cyanide and oxalate contents were relatively low ranging from 0.09-1.28mg/100g in cyanide where Star of David had the maximum value while it was 0.51-0.88mg/100g in oxalate where Silver Queen had the maximum value though not significantly different from other varieties ($P>0.05$). Phytic acid content was the highest anti-nutrient quantified and it differed significantly among the varieties ($P<0.05$) where it was lowest in Silver Queen (46.7mg/100g) while the highest amount was found in Clemson spineless (64.8mg/100g). Figure 2 shows that the overall mean phytic acid of 62.3 mg/100g was 49 times higher than oxalate and 59 times higher than cyanide in okra

The anti-nutrients found in the okra seeds are important for discussion. All the values reported in this study are below the permissible limits of WHO (WHO, 2015) for anti-nutritional factors and therefore, the three varieties are healthy for consumption. Oxalate and cyanide were present but in negligible amount. These factors are part of the normal phytochemical compounds present in plants as products of metabolic biosynthetic pathways, although they interfere with absorption of nutrients leading to deficiencies in essential minerals (Khomsug *et al.*, 2010; Roy *et al.*, 2014). The most important of all the nutrients studied in this work is the phytic acid component being the highest quantified (about 49-59 times higher than the amount of oxalate and cyanide respectively in okra seeds), although it was below regulatory limits. Significant variation was also observed in the level of phytic acid among the three varieties and this may be due to genetic factors (Adiger *et al.*, 2011; Bisht *et al.*, 2015; Ford, 2018).

From all indications, there are needs to reduce the level of phytic acid in the seeds. This is important to ensure that the body absorbs the rich nutrients present in the seeds maximally without interference from phytic acid (Roy *et al.*, 2014). Although the mineral component was not included in this study, there are claims that phytic acid reduced the amount of minerals such as zinc and iron in foods needed by the body system (Roy *et al.*, 2014). This study found that the Silver Queen variety that came top in proximate analysis also emerged the best in the anti-nutritional study being the lowest in phytic acid content. It therefore suggests that this variety possibly possesses certain genes that drive the production of useful biochemical compounds as well as those that suppress the production of others. This is because the production of all forms of active ingredients and metabolites in plants are regulated by genes (Bisht *et al.*, 2015; Ford, 2018).

Ash had high positive correlation with Fibre (0.958), Protein (0.995), and Oxylate (0.697). Moisture had negative correlation with all variables except Ash) as obtained in Table 3. Fibre had a positive correlation with Ash (0.958), Protein (0.981), and Oxylate (0.873). Phytic acid had a negative correlation with all variables except Carbohydrate (0.840). It shows that carbohydrate level positively influences phytic acid level in okra seeds.

Therefore, the high amount of carbohydrate reported in this study may account for the escalated quantity of phytic acid. There are many options for breeders in this regard. The carbohydrate level may reduce or varieties that have low phytic acid content may be bred. This suggestion conforms with some reports where food crops are improved to reduce allergies (Siemonsmo, 2012; Singh and Oswalt, 2015).

The negative relationship established between other nutrients and phytic acid is a welcome development because when nutrients like protein, lipids, fiber and moisture are improved upon, the phytic acid level may reduce drastically. Okra seeds are rich sources of nutrients; hence consumers should not discard them in meal preparation. The Silver Queen variety should be popularized for cultivation and consumption for its additional values. Further studies are needed to evaluate all types of anti-nutritional factors in okra and other vegetables. Breeding efforts are needed to improve the nutrition of okra and reduce the anti-nutrients.

Table 1: Proximate composition in three varieties of okra

Varieties	Moisture (%)	Ash (%)	Fiber (%)	Lipid (%)	Protein (%)	Carbohydrate (%)
SQ	8.33±0.33	8.17±1.59	11.17±1.45	7.00±0.29	22.93±0.10	45.57±1.44
SD	11.33±0.17	6.40±0.76	8.00±0.29	6.70±0.15	22.52±0.06	43.15± 0.37
CS	11.17±0.60	7.07±0.34	8.33±0.17	7.03±0.09	22.64±0.06	39.51±0.58
Grand mean	10.28±0.97	7.21±0.52	9.17±1.01	6.910±0.105	22.69±0.12	42.74±0.99

Carbohydrate: χ^2 (Variety Vs Carbohydrate content) = 0.435, P=0.804 (P>0.05)
Moisture: χ2 (Variety Vs moisture content) = 0.55, P=0.758 (P>0.05)
Ash: χ2 (Variety Vs Ash content) = 0.22 P=0.895 (P>0.05)
Fibre: χ2 (Variety Vs fibre content) = 0.0663 P=0.718 (P>0.05)
Lipid: χ2 (Variety VsLipid content) = 0.01 P=0.995 (P>0.05)
Protein: χ2 (Variety Protein content) = 0.004, P=0.0998 (P>0.05)
SQ=Silver Queen, SD= Star of David; CS=Clemson Spineless

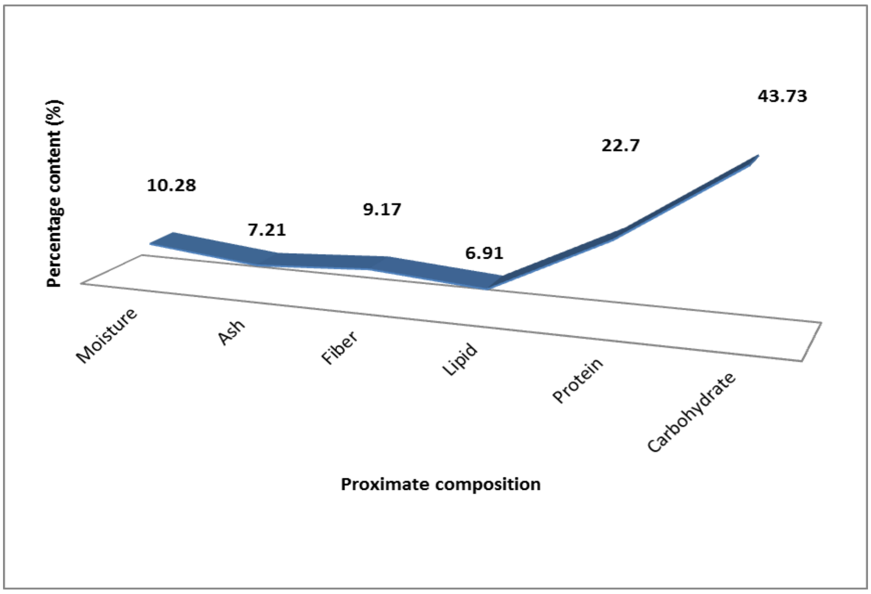

Figure 1: Graph showing the proximate composition as express in percentage

Table 2: Anti-nutritional factors in three varieties of Okro

Varieties	Cyanide (mg/100g)	Oxalate (mg/100g)	Phytic acid (mg/100g)
Silver queen	$0.97^{b}\pm0.04$	$0.88^{a}\pm0.13$	$46.65^{c}\pm0.71$
Star of David	$1.28^{a}\pm0.01$	$0.66^{a}.\pm0.13$	$75.49^{a}\pm0.74$
Clemson spineless	$0.09^{b}\pm0.01$	$0.51^{a}\pm0.07$	$64.8^{b}\pm0.74$

F (Cyanide content) = 74.60, P= 0.00 ($P<0.05$)
F (Oxylate content) = 2.71, P=0.145 ($P<0.05$)
F (Phytic acid content) = 401.52, P=0.00 ($P<0.05$)
Means that do not share a letter are significantly different.

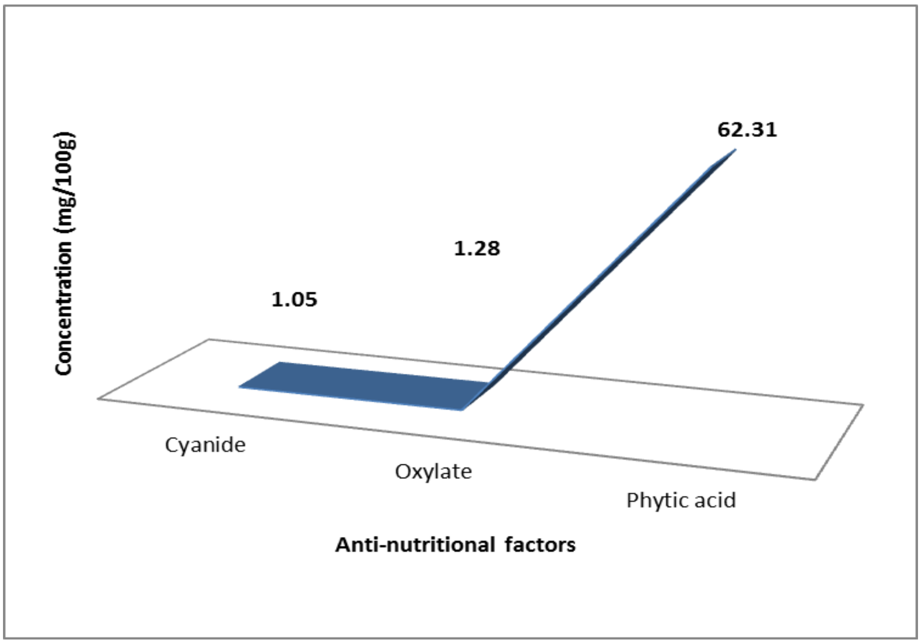

Figure 2: Anti-nutritional factors in three varieties of Okra

Table 3: Pearson's Correlation Matrix

	Ash	Moisture	Fibre	Lipid	Protein	Carbohydrate	Cyanide	Oxylate	Phytic acid
Ash	1								
Moisture	-0.941	1							
Fibre	0.958	-0.998	1						
Lipid	0.735	-0.462	0.511	1					
Protein	0.995	-0.969	0.981	0.667	1				
Carbohydrate	-0.845	0.613	-0.657	-0.984	-0.790	1			
Cyanide	-0.670	0.379	-0.430	-0.996	-0.596	0.963	1		
Oxylate	0.697	-0.899	0.873	0.027	0.763	-0.205	0.065	1	
Phytic acid	-1.000	0.944	-0.961	-0.729	-0.996	0.840	0.663	-0.704	1

CONCLUSION

Okra seeds are rich in balanced nutrients and therefore of high nutritional values. Although the three varieties have good nutritional qualities, the Silver Queen variety was the best being high in carbohydrate, protein, fiber and moisture together with minimal level of anti-nutrients. It is important to improve all the varieties for certain nutrients such as protein and lipids so as to reduce the phytic acid level based on the results obtained. This information is important to the breeders, the growers, consumers and the general public.

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Evaluation of Fenugreek (*trigonella foenum graecum*) Genotypes Against Powdery Mildew (*Erysiphe polygoni*) in Ethiopia

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Abstract:
Background and Objective: Ethiopia have suitable environmental condition for fenugreek production, it grows under agro ecologies ranging from 1800 to 2300 m.a.s.l. Fenugreek is mainly used as an herb (leaves) and as a spice (seed), medicinal value and also as nitrogen fixation and soil enrichment. Perhaps Powdery mildew caused by Erysiphe polygoni is an economically important disease especially during the flowering and pod formation stage on the fenugreek and causes significant loss in grain quality as well as quantity. Hence the present study done with the objectives to identify source of resistance among 100 Ethiopian fenugreek materials for further resistance breeding program and for identifying high yielding genotypes to ensure sustainable production and productivity. Materials and Methods: The Current study was done using lattice design during 2021/2022 cropping season under natural epidemic conditions to evaluate the fenugreek genotypes obtained from Ethiopian Bio diversity Institute at kulumsa, South Eastern Ethiopia. Result: The current evaluation of fenugreek genotypes against powdery mildew results identified genotypes namely 28601 and 29561 showing moderately resistant reaction and forty-three genotypes as moderately resistant type. The remaining 55 genotypes showed susceptible reaction. The Present result also identified the genotypes,31100, 29561 and 212775 were with lower rAUDPC which could cross with 238247 and 9239 which are relatively high yielder. Conclusion: The study results recommended having a crossing program between genotypes 31088,237983 and 20428 and 35190 and 31087 for improved resistance and high yielder. Contemporary results suggested genotypes with moderately susceptible and moderately resistant material for further resistant breeding considering other physio chemical evaluation quality mechanisms including evaluating under greenhouse condition.

Keywords: Fenugreek, Powdery mildew, evaluation, resistance, genotypes, field.

INTRODUCTION

The cognitive content of yield losses is the primary program in an effort to combat hunger, raise income and improve food security mainly in the poorest countries of the world. Crop yield losses have an impact on food security, economic development and the environment. The exact causes of yield losses vary throughout the world and are very much dependent on the specific conditions and local situation in a given country. Ethiopia has diverse agro-ecologies which enable it to grow various crop species. Although agriculture is the backbone of the Ethiopian economy, annual agricultural production and productivity growth have not been commensurate with the annual population growth rate. The production and productivity of different crops in all agro-ecologies are constrained by several biotic and abiotic stresses, which can vary across agro ecologies and from place to place within an agro ecology. Several factors including climate change which aggravates pest problems and diverse agro-ecologies that need diverse management options and low level of access to improved crop production and protection contribute to the poor performance of Ethiopian agriculture. These factors slow the growth of agricultural production in general and food grain production in particular; eventually contributing significantly to food insecurity. Therefore, the incorporation of yield-increasing technologies like disease resistant varieties and other control measures, which are environmentally safe are all crucial points to be taken into consideration.

In Ethiopia, the performance of the agricultural sector has been constrained by biotic and abiotic factors, which in turn affected its contribution to yield reduction. Powdery mildew is one of the major biotic constraints causing losses in quantities and qualities in fenugreek production. Fenugreek or *Trigonella foenum-graecum L.*, is an annual plant and is an extremely important crop for Ethiopia's economy and the country has ideal natural conditions for its cultivation. It is important in an incapacitating economy as a spice cash crop, medicinal value and also as nitrogen fixation and soil enrichment. Fenugreek now covers around 34,603.81 hectares in Ethiopia, with an average national productivity of 1.3 t/ha, which is lower than what can be obtained (1.7 t/ha) with proper management techniques[1].Due to inadequate agronomic practices, diseases (such as powdery mildew and wilt) and insect pests (borer) in the research area as well as other agro-ecological zones of Ethiopia, the large yield difference linked to the lack of stable, high yielding and disease resistant genotypes[2]. According to the study done on the crop by Fikreselassie[3]**,** powdery mildew had a significant effect on the number of seeds per pod and thousand seed weight yield parameters. Powdery mildew was prevalent on fenugreek in the central highlands of Ethiopia with an incidence of about 95% and severity ranges from 20 to 80%[4]. The disease downy mildew of fenugreek was reported from Algeria, India, Pakistan and the United Kingdom but the first report of its occurrence was from California the United States and India. It is observed that the disease commonly occurs during February and March. The pathogen causing downy mildew of fenugreek was identified as *Peronospora Trigonella Guam*[5].

The symptoms of downy mildew are quite distinct from the other diseases. The axial surfaces of the leaves showed yellow patches or small chlorotic spots which appeared at the margins often. The axial surface of the leaf showed white cottony mycelium growth which often appears as grayish violet. The disease also causes stunted growth of the plant[6].Though powdery mildew is the number one yield constraining disease of the crop; less focus is given to the management of the disease in fenugreek. In resistant breeding for powdery mildew management in fenugreek, Bélanger[7] reported that a mildew resistant fenugreek variety Chala (FG-47-01) has been developed and released for mildew risk areas. As host plant resistance is the best and most economical for plant disease management, investigations of moderately resistant genotypes are paramount in breeding for disease resistance. Therefore, a wide gap in yield is attributed to a lack of stable, high yielding and disease resistant genotypes for different agro ecological zones of Ethiopia including the study area, hence the study was designed with objective of evaluation and recommendation of potential sources of resistance fenugreek genotypes against powdery mildew under field condition.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted at Kulumsa Agricultural Research Center, South Eastern Ethiopia from July 2021 to February 2022.The representative agro ecology of Kulumsa is characterized as waterlogged Vertisols[8].

Planting Materials

One hundred fenugreek accessions along with one local check were used for this study. The majority of the accessions represent the national collection from major growing regions of Ethiopia, mainly obtained from Ethiopian Biodiversity Institute (EBI),while one local variety was obtained from a local farmer. The total lists of planted one hundred genotypes are retained along with the Author.

Experimental Design

The experiment was laid out in alpha lattice design in three replications. The plot size was a single row of 1.5 m long and spacing of 0.30 m between rows and 0.10 m intra-row spacing. A replication holds 4 blocks and contains 100 entries (genotypes) including local checks. Spacing between sub blocks was 1 m and between the two replications was 1.5 m. Therefore, the total experimental area was 11m X 30.3m = 333.3 m2. The seed and fertilizer rates were as per the national recommendation for the crop. i.e., 25 kg /ha of seed, 100 kg for NPS(Nitrogen(N), Phosphorus(P205) and Sulfur (SO3)) and 50 kg UREA /H2NCONH2 per hectare. Half the rate of UREA and full dose DAP were applied at planting time and the second half of UREA was applied on the second round.

Data Collected and Measurements

Progress of disease development in the plants was observed five times during the epidemiological period. Disease assessments were made by observing the plants that were planted on the plot or row and recorded as diseased and healthy. Disease incidence and severity were taken as Danielsen & Munk, standard procedure[9].Disease Incidence (DI): is the number of infected plants over the total number of plants per plot and Disease severity (DS) is defined as the affected leaf area, including the lesion and associated chlorosis (non-green area) as a percentage of total leaf area.

The severity score from five selected plants was converted into percent severity index (PSI) for analysis using the formula suggested by Jenkins & Wehner[10].The disease severity indexes obtained from different assessment periods were used to calculate the Area under the Disease Progress Curve (AUDPC-% day) of the recording period. Area under the disease progress curve (AUDPC) was calculated for each genotype[11].

Disease Assessment

Starting from when tillers first appeared and continuing at intervals of five days throughout the entire season, the severity of powdery mildew was measured on five randomly selected tagged plants in a plot. According to a 0–9 rating scale, the disease was scored based on the percentage of leaf area that was infected (Jenkins and Wehner,1983).0 = No illness 1= a few tiny leaf lesions (0–3), 2= a few leaf lesions (3-6) but no stem lesions,3-6 superficial stem lesions or few lesions on few leaves, 5= 25-50 Few well-formed leaf lesions or growing stem lesions,4= 12–25 few well-formed leaf lesions or superficial stem lesions 6= 50-75 a plant with more than 50% of its leaves missing, several huge leaf lesions, deep stem lesions with profuse sporulation.

Phonological Parameter

Days to 50% flowering, number of days between planting and 50% flowering of a row was recorded. Days to 95 % physiological maturity: was calculated as the number of days between planting and 95 % physiological maturity in an experimental unit.

Yield and Yield Components

Average number of pods counted from the five plants that were randomly chosen. The average number of primary branches (branch lets that extend from the main stem) was calculated for five randomly selected plants. Plant height (PH) in cm. The height of plants was measured from the ground up to the tip of the main stem at 90% physiological maturity. The difference between the days to maturity and the days to flowering (DM-DH) was used to compute the grain filling period (GFP). The average number of nodes from the five studied plants was used to calculate the number of nodes per plant (NNPPl). The average of the total number of pods on five randomly chosen plants is used to calculate the number of pods per plant (NPPPl).

Statistical Analysis

Analysis of variance (ANOVA) made using SAS version 9.2[12]. When ANOVA indicate significant difference among treatment means. Mean comparisons were carried out using the least significant difference (LSD) at a 5% level of significance. Correlation analysis was made to analyze the relation of the disease parameters to yield parameters and principal component analysis was made to group the genotypes.

RESULTS AND DISCUSSION

Disease Development and Genotype Reaction

The study revealed that there was disease reaction variation among Ethiopian fenugreek landraces. Different scholars also found that there is a variation among fenugreek genotypes in reaction to powdery mildew pathogen. The evaluated genotypes fall into four disease reaction classes namely resistant, moderately susceptible, susceptible and highly susceptible against the powdery mildew pathogen according to Jenkins and Wehner rating scale[10].Among the hundred genotypes evaluated two genotypes namely 28601 and 29561 exhibited resistance reactions against the powdery mildew pathogen with the mean disease severity of 9.49 and 7.19 respectively. Similarly, forty-three of the genotypes showed moderate resistance, fifty-four were susceptible and one genotype showed a highly susceptible disease reaction with mean disease severity of 69.92.

The current results indicated that there were high variations in disease development between the moderately resistant and susceptible genotypes which reveals resistant genotypes potentially reduce the disease severity while disease severity on susceptible genotypes. This finding was in line agreement with the finding of Raje[13], who reported that there was a heavy incidence of powdery mildew in susceptible check while less incidence in a moderately resistant material. Apart from powdery mildew other fenugreek disease like; Cercospora leaf spot and rust were recorded and considered during the experiment.

Analysis of Variance/ANOVA

ANOVA result for agronomic and disease parameters also revealed that different fenugreek genotypes responded differently to the infection of powdery mildew (Table 1). The results showed that AUDPC and all agronomic parameters except plant height and number of primary branches per plant (NPBPPl) were significantly different among tested genotypes. The analysis of variance showed that there is no significant difference among genotypes concerning plant height and number of primary branches per plant. This might be related to the late coming of the pathogen at which the plant grows to its optimum height and primary branch and the two traits were similar in fenugreek genotypes. Typical powdery mildew symptoms of infection were observed beginning from sixty-two days post-germination. The symptoms began from the lowest leaf among the plants. The powdery mildew appeared as small white powdery spots on the lower and upper surfaces of leaves. The powdery mildew progresses to other leaves as the plants grow. These were observed virtually on all the leaves of the plants. Benagi[15] screened 110 lines of fenugreek for resistance to Erysiphe polygoni, Rhizoctonia solani and Fusarium oxysporum in Harry. None of the genotypes was completely resistant to all three pathogens However, GP75, GP82, GP94, GP and PEB were the moderately resistant lines and lines are significantly different in the yield and yield component parameters.

Table 1: Growth and phonological parameters of evaluated fenugreek germplasm reactions for powdery mildew (*Erysiphe polygoni*) under field conditions.

	DE	DF	DM	GFP	PL	NPBPPl	NSBPPl	NNPPl	NPPPl
min	5.33	44.33	130	82	6.47	3.1	0.47	13.07	3.13
max	8	49.33	135.67	90	9.63	5.4	3	41	15.73
mean	6.67	46.86	132.24	85.38	8.27	4.03	1.94	28.05	10.2
Cv	13.33	3.06	0.95	1.9	6.5	11.63	5.45	18.89	26.2
LSD	1.43	2.31	2.03	2.61	0.87	4.46	0.35	4.31	0.59
P value	0.03	0	<0.0001	0.02	<0.0001	0.55	<0.0001	<0.0001	<0.0001

Key: PL= Pod length, NPBPP= number of primary branches, NSBPP= Number of secondary branches, NNPPl=Number of nodes per plant, NPPPl =Number of pods per plant, DE= Days of emergence, DF= Days to 50% flowering, DM=Days to maturity, GFP=Grain filling period, LSD=Least significant difference.

Correlation Analysis

Pearson's correlation coefficients between possible pairs of agronomic traits and disease parameters were tested using SAS software 12. The results showed that the correlation between most of the yield and yield components in fenugreek is positive and significant(Table2).Seed yield per hector had positive and significant correlations with all paired yield component traits except pod length. The result revealed that genotypes with better (longer) grain filling periods are better in their seed yield and the plants bearing more nodes per plant, a greater number of pods per plant and a greater number of seeds per pod produce more seed yield. Indeed, genotypes with better hundred seed weights had higher above-ground biomass and seed yield per hectare. Thus, selection for better yield component traits will bring about a definite improvement in above-ground biomass and seed yield. The trait Seed Yield per hectare is non-significant with disease parameters, an area under the disease progress curve (AUDPC) and disease progress rate. The disease parameters AUDPC and disease progress rate were non-significant with most yield component parameters except grain filling period and number of seeds per pod. This result revealed that the tested genotypes were diverse in their yield traits and yield traits are genetically different because of the disease effect. Generally, this study revealed that resistant genotypes identified in this study significantly reduced the disease parameters but low yielder. This phenomenon happens in non-elite resistant material because they mobilize most of their genetic resource for disease or stress response rather than yield response. As powdery mildew is a series disease in fenugreek the resistant genetic materials identified in this study are important for cross-breeding with elite high-yielding genotypes.

Table 2: Correlation between different agronomic traits and final disease reaction to powdery mildew disease on fenugreek genotypes

	GFP	PL	NNPPl	NSPP	SYPPl	HSW	AGBM	HI	rAUDPC	DPR
PL	0.106ns									
NNPPl	0.211*	0.353**								
NPPPl	0.237*	0.348**	0.820**							
NSPP	0.356**	0.253*	0.205*	0.188ns						
SYPPl	0.168ns	0.137ns	0.342**	0.213*		0.511**				
HSW	0.193ns	0.135ns	0.107ns	0.033ns		0.515**		0.480**		
AGBM	0.274**	0.249*	0.237*	0.217*		0.799**		0.695**	0.665**	
HI	0.000ns	-0.107ns	0.143ns	0.156		-0.183		0.331**	0.037ns	-0.181ns

Key: NSPP = number of seeds per plant, SYPPl= Seed yield per plant, HSW=Hundred seeds weight in gram, SYPH = Seed yield per hector, AGBM = above-ground biomass, HI= Harvest index, and rAUDPC= residual Area under disease progress curve, *=significant @ p<0.05; **=significant @ P, 0.01; ns=non-significant.

Cluster Analysis

Hierarchical clustering of the average linkage method with squared Euclidean distance was performed using SAS or/ and MINITAB14 software (MINITAB,2003). The distances between clusters were calculated using the average linkage method of squared Euclidean distance. The average linkage Euclidean distance technique of clustering produced a more understandable portrayal of the 100 accessions by grouping them into six clusters, whereby different members within a cluster are assumed to be more closely related in terms of the trait under consideration with each other than those members in different clusters. Similarly, members in clusters with non-significant distances are assumed to have more close to each other than they are with those in significantly distant clusters. In this study, the hundred genotypes were grouped into five clusters (Table3). The maximum distance i.e., ED=9.642 was found between cluster 3 and cluster6. On the other hand, minimum inter-cluster distance (3.700) was recorded between clusters 2 and 4 indicating their genetic related. While the highest intra clusters some of square (947.379) were recorded in cluster2 which consists of 74 genotypes.

Table 3: Clusters of 100 fenugreek genotypes into different diversity classes.

Cluster-1	Cluster-2					Cluster-3	Cluster-4	Cluster-5	Cluster-6
G1	G2	G7	G74	G75	G35	G3	G12	G23	G88
G15	G55	G8	G56	G33	G84		G14	G97	
G39	G73	G40	G62	G50	G78		G95	G100	
G91	G58	G86	G11	G71	G80		G48	G98	
G64	G69	G72	G45	G6	G77		G52		
G66	G59	G85	G22	G60	G81		G49		
G34	G93	G24	G83	G70	G20		G18		
G87	G65	G27	G45	G82	G37		G68		
G94	G13	G42	G25	G26	G28		G31		
	G46	G43	G30	G67	G92		G51		
	G93	G47	G32	G89	G99		G63		
	G5	G10	G96	G61	G54				
	G38	G17	G41	G21	G4				
	G19	G16	G44	G51	G9				
	G90	G36	G76	G79					

G= Genotype

Cluster 1: It consisted of 9 genotypes which were collected from Oromia and Amhara regions. There is a genetic distance between cluster one and cluster two. Members in this cluster laid on intermediate value in all the traits under consideration.

Cluster 2: It consisted of 74 genotypes, which were early in days to flowering, intermediate in biomass yield, number of pods and seeds per plant and number of seeds per pod. Among these clusters, the genotype/ accession, 35190 is a high yielder. Accessions in this cluster also exhibited lower with hundred seed weight, seed yield per plant harvest index and 1 accession exhibited resistance and 17 the remaining exhibited moderately susceptible as well as susceptible to powdery mildew disease.

Cluster 3: It consisted of 1 genotype characterized by late days to flowering; low seed and biomass yield and a number of seeds and pods per plant high in hundred seed weight. Accessions in this cluster also exhibited an intermediate, number of seeds per pod harvest index and resistance to powdery mildew disease.

Cluster 4: It had 11 genotypes which exhibited early growth periods, short days to flowering; low in hundred seed weight and intermediate in both biomass yield and number of pods per plant. Among these clusters, the accession,237935 showed a relatively high mean. These accessions also exhibited intermediate, seed yield per plant, number of seeds per pod and number of seeds per plant, harvest index and resistance to powdery mildew disease.

Cluster 5: It consisted of four genotypes, collected one from Bale Ginir, one from East Gojam, and one from south Gonder. The accessions under this category were relatively inferior in most of the traits investigated. It was characterized by intermediate days to flowering; exhibited lowest in all traits under study except hundred seed weight, harvest index and moderately susceptible to powdery mildew.

Cluster 6: It consisted of one genotype from Tigray. It was found to be the most superior accession regarding the traits studied. This accession was characterized by a low hundred seed weight and harvest index. However, this particular accession also required a longer period to maturity, characterized by intermediate seed and biomass yield per plant, number of seeds and pods per plant and seeds per pod and moderately susceptible response to powdery mildew. In general, the differences between the clusters were mainly attributed to the variation in all traits. Other traits such as days to flowering, biomass yield and number of seeds per plant have contributed equally well for cluster castellations.

CONCLUSIONS

The resistant and moderately susceptible genetic materials identified in this study can be used for further exploitation of the genes for disease resistance breeding in the future. I highly recommend that those materials viz 28601 and 29561 found resistant to powdery mildew, disease be used as germplasm to broaden the genetic base of fenugreek for sustainable production in the country in general. However, further evaluation of the materials under optimum disease pressure including evaluation of the materials under greenhouse conditions is recommended.

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