2. **Brief Introduction:** *Jatropha curcas* is a large soft wooded deciduous multipurpose tree of 4-7 meter height and belongs to family *Euphorbiaceae* it shows vigorous growth in early periods. It flowers in hot and rainy season and fruit in winter in field condition it may produce seeds with seed yield as high as 1200 kg/ha/year after 5 year of plantation (Jones and Miller, 1992).

2.1 **Average number of seeds per kg:** The coefficient of variance helps in comparing variability among different sources which was found to be maximum (7.31%) and minimum (1.03%) for fruit diameter in S_{21} and S_{5} respectively whereas for number of seeds per fruit it was 14.52% (S_{5}) and 1.87% (S_{9}) respectively, reported by Williums (1967) and Kandya (1978). The seed length, seed width and seed thickness were highest in S_{14} (18.53mm), S_{24} (11.24mm) and S_{3} (9.21mm) respectively, reported by Shiv Kumar and Banerjee (1985).

2.2 **Average weight of seed per kg:** The seed weight record has been reported highly positive significant (P<0.01) correlation with germination power (GP), power value (PV) and plant and significant (P<0.05) correlation with mean days germination (MDG), germination value (GV) and collar diameter, reported by Langdon (1958), Williums (1967) and Kandya (1978). All the germination indices and growth traits registered highly positive and significant (P<0.01) relationship exist with each other. Seed length and seed width (P<0.01) both seed traits have weak or negative correlation with germination and growth traits, by Shiv Kumar and Banerjee (1985).

2.3 **Dimension of the seed:** In general, all the seed sources with height and weight except Pusa sources (S_{1}) were recorded earlier relatively higher germination value and growth traits. This confirms the dependence of this trait on the seed weight and gives the basis for consideration of seed weight in delineating and understanding the geographic variation, it may be possible due to cumulative effects of both internal and
external conditions prevailing during the process of seed developments earlier (Harper et al., 1970). The significant correlation among seed weight and seed uniformity of germination has been reported by Shiv kumar and Banerjee (1985). Similarly, Langdon (1958), Williams (1967) and Kandya (1978) have earlier reported seed size as an important character to regulate germination and subsequent seedling growth in Pinus elliottii, Oocarpa and Vicia dasycarpa.

2.4 Average moisture content of the seed: Generally 20 ºC and 10 ºC reflect significant difference (Purohit et al., 1982) in the moisture content of seeds. The storage temperature of seeds at 10 ºC were found maximum for germination percentage, on the other hand the minimum values were exhibited by seeds stored at room temperature. The germination ability of Jatropha curcas seed was enhanced at 10 ºC may be ascribed to the slow rate of metabolic processes, whereas at higher temperature the germination ability of seed is lost rapidly due to rapid metabolic activity and rapid loss of moisture content of seeds (Purohit et al., 1982).

Although low temperature is generally preferred in order to reduce ageing and prevent insect and fungal activity. The better germinative energy of seeds stored at 10 ºC may be ascribed to the slow rate of biological processes compared to 20 ºC and room temperature (26.33 ºC) (Gautam et al., 2005). The seed stored at high temperature i.e. at ambient condition exhibited progressive deterioration and partially decrease in the speed of germination during seed storage. This may be due to decrease in moisture content during storage and hence invasion of fungi along with high respiratory activity in seed leading to degradation.

Gupta and Raturi (1975) observed that moisture content of seed stored under high temperature reduced quickly to maintain equilibrium moisture content with surrounding. Low temperature slowing down the rate of seed deterioration during storage, and prevent excessive drying of seeds (Gupta and Raturi, 1975).
2.5 **Seed viability:** The highest percentage germination was observed p-6 (64.00%) followed by p-5 (63.00%) and the lowest was recorded in p-12 seed source (24.53%). But p-5 seed source showed significantly highest germinative energy followed by p-20 (45.33%) and p-6 (42.16%) respectively whereas p-11 (9.77) had lowest germinative energy (Zhang and Cui, 1993; Jaiswal and Kanauji, 1994). Germination percentage reflected, highest range (24.53-65.00%) with mean value (45.70%), coefficient of variation (6.58%) and lowest range in germinative energy (9.77-45.69) with mean value (29.43) and lowest coefficient of variation (5.43%) (Cambell, 1979). The maximum phenotypic coefficient of variation and genotypic coefficient of variation were for germinative energy (31.59 and 31.12%) respectively, while minimum phenotypic coefficient of variation and genotypic coefficient of variation were observed for germination percentage (27.10 and 26.29%) respectively. Maximum environmental coefficients of variation were estimated for germination percentage (6.58%) and minimum environmental coefficient of variation in germinative energy (5.43%) presented.

Similar type of variation was observed by Kumar *et al.* (2006) in *Jatropha curcas* and Khosla *et al.* (1988) in *Pinus roxburghii*.

2.6 **Microbial flora on the seed coat:** Recently Zakaria *et al.* (2010) have isolated bacteria from a digester treating palm oil mill effluent and was able to produce 9.8g/l of poly-hydroxy-alkanoate (PHA). Rawte *et al.* (2002) have isolated 337 PHA producing bacteria from marine and mangrove microflora on the mid west coast of India. Wang *et al.* (2009) have isolated *Psychrotrophic* bacteria from the abyssal-benthic sediments in Bohai sea which was able to synthesize poly-hydroxy-alkanoate polymer consisting of mainly 3-hydroxydecanoate and 3-hydroxydodecanoate, (Redzwan *et al.*, 1997).
2.7 Potting Mixture: The effect of soil mixture on the growth and quality of seedling of various tree species has also been reported by many researchers (Ginwal et al., 2002; Tiwari and Saxena, 2003; Srivastava et al., 2008). Among all the soil mixtures, seedling of *Jatropha curcas* indicated higher growth and dry weight in the mixture of soil, sand and Farm yard manure (FYM) in 1:2:2 and 1:1:2 ratios. It was further observed that increasing FYM in all combinations of soil mixture led to an increase in the seedling growth, which may be due to the fact that the water holding capacity of FYM is always greater than the sand and soil alone or in combination (Nayital et al., 1995). In addition the FYM also improves physical status of soil and nutrient uptake (Kaberathumm et al., 1993). Thus the increase in FYM in the soil mixture favors the seedling growth observed for *Acacia nilotica* (Singh et al., 2000), *Albizia procera* (Ginwal et al., 2004), *A. amara* (Handa et al., 2005) and *Picea Simithiana* (Lavania et al., 2007). However, the increase of sand in the soil mixture beyond 1:2:2 ratio of soil, sand and FYM was not much beneficial to the growth of *Jatropha curcas* seedlings. This is in conformity with the findings of Tiwari and Saxena (2003) for *Dalbergia sissoo* seedlings. Higher proportion of sand in the soil mixture reduces the water holding capacity and thus inhibits the seedling growth. Further, the soil mixture with high proportion of sand lacks aggregation due to which the container soil gets dispersed at the time of planting leaving the seedling naked and may in poor survival. Such soil promotes the leaching of nutrients (Lavania et al., 2007) into poor growth of seedlings.

Several workers (Koul et al., 1995; Singh et al., 2001) have advocated the balance and complementary use of fertilizers to maximize the growth and biomass production of seedling nursery. In the present study the application of NPK fertilizers @ 100:75:75 mg per seedling in soil indicated maximum improvement in growth.
2.8 **Soil analysis:** The increase in growth of plants with the application of FYM along with inorganic fertilizers may be attributed to a slow release of macro as well as micro-nutrients from FYM. Besides, application of organic manure to soil improves the physical properties (like water holding capacity, bulk density, soil structure, etc.) of the soil and influences the plant growth positively. Significant effect of inorganic nutrients and organic manure on the growth of seedling of *Acacia nilotica* and *Albizia procera* has been reported by Singh *et al.* (1995) and Deswal *et al.* (2001), respectively.

Further increase in the dose of inorganic nutrients with and without FYM did not increase the growth of plants significantly. This might be attributed to accomplishment of nutrient requirement of plants as the organic carbon and available P of upper top soil layers (0-15 and 15-30 cm) were medium in their status and major mass of root system concentrates in these layers (Singh, 1994). Moreover, the site which has loamy to sandy loam texture where the availability of applied nutrient is not hampered and plant can take up those nutrient elements from the applied inorganic fertilizer and FYM.

2.9 **Soil microflora of soil sample:** Poly-hydroxy-alkanoate (PHA) degrading microflora have been reported by Manna *et al.* (1999) and the degradation of Poly-hydroxy-butyrate (PHB) by streptomycetes where rate of degradation increased with increase of PHB in the medium.

Hoang *et al.* (2007) isolated 135 actinomycetes from upstream and downstream region of Touchien River in Taiwan which can degrade PHB. Microbes which can degrade other polyesters have also been isolated.

2.10 **Seed germination studies:** Germination percent age and germinative energy differ significantly due to seed size. Highest germination percent (84.40%) and
germinative energy (56.80%) were recorded in S₁ (large seeds) whereas minimum germination percent (40.40%) and germinative energy (19.00%) were recorded in S₃ (small seeds). Seed size has significant effect on seed germination (Baldwin, 1942). Generally larger seed germinate fast and more comparatively the smaller one due to more endosperm nutrient pool. Hence variation in germination may be due to difference observed in seed dimensions. In general it was observed that the germination percentage increase with the size and weight of seed. However, seed size does not vary significantly with time taken to initiate germination. Baig (1982) and Jaswal (1992) e.g. in Grewia optivas grading is a useful practice for the production of evenly growing crop of seedlings in the nursery. Gurunathan (2006) has also reported the usefulness of grading the seeds of Jatropha curcas based on size and weight. Jatropha curcas shows wide variations in its seed size hence seeds were graded into three size classes. Maximum proportion of seeds was in medium seed size class in Acacia nilotica, Albizia lebbek and Dalbergia sissoo (Khera et al., 2004).

Germination behavior has been observed better in large and heavier seed than the smaller and lighter ones by earlier workers for Spindus trifoliatus (Girish et al., 2001), Bassia longifolia (Suresh et al., 2003), A. Catechu, A. nistotica (Khera et al., 2004) and for Pinus wallichiana (Ghildiyal and Sharma 2005).

2.11 Seedling growth studies: It has been observed that raised seedbed of soil +FYM mixture (3:1) markedly improve germination percentage viz. final plant stand, plant height, number of branches per seedling, collar diameter, root, total length, leaf, per seedling in different plant parts like root, shoot and leaf over other growth media, followed by raised seedbed of soil alone. The lowest values of these parameters were recorded in polythene bags filled with soil +FYM mixture (3%) by Bahuguna and Lal (1990), Singh et al. (1990), Gupta (1992) and Tiwari and Saxena (2003). This may be
ascribed that variation in growth of seedling under different growth media owing to
variation in temperature as given in Jatropha curcas exhibits epigeal type
germination. Growth media used for propagation should be loose and friable for easy
eremergence of coleoptiles. Stagnation of water near root zone at the time of
germination may have higher incidence of fungal infection which is induced the
variation in quality and vigor of seedlings (Chen and Manu 1999; Kokou et al., 2001;
Seiwa et al., 2002).

2.12  Morphology of Jatropha curcas Linn.:

2.12.1 Number of branches per tree: The number of fruitless branches/tree (21.44)
respectively whereas minimum genetic gain was in the characters seed viability (1.54)
and germination energy (2.08).

2.12.2 Number of fruits per branch: The information about number of fruits/branch
will suggest the health of the tree. The number of fruits/branch (23.44).

2.12.3 Number of fruits per tree: The number of fruits/tree (54.56) and oil content
on seed basis (11.14). Genetic gain was maximum in oil content on seed basis (50.07)
followed by number of seeds/tree (35.22), number of fruits/tree (34.99).

2.12.4 Number of seeds per tree: The heritability was maximum in oil content of
kernel basis, number of fruits/tree and number of seeds/tree (0.99). Higher value of
genetic advance was noticed in number of seeds/tree (60.08).

These estimates indicate that the above three characters have good
opportunities for practicing selection which is the basic tool of plant improvement
(Lantz, 1975). The adaptation strategies along the seed source have also been
suggested for tree species by Rehfeldt (1983).

The factor comprises the seed traits and is a combination of number of
seeds/tree, seed length, and seed width and seed weight. Seed weight signifies the
combination of number of fruiting branches and fruitless branches. Result of such
work have been reported by Zhang and Cui (1993) and Jaiswal and Kanaujia (1994).
2.13 Oil content percent: Moreover, the oil content extracted from seeds collected at different fruit maturity stages from wasteland showed maximum oil content than the seeds collected from cultivable land. Diwaker (1993) reported that *Jatropha curcas* species grows well in wasteland/degraded lands, which may be the factor responsible for high oil content.

Similar ranges for oil content on kernel weight basis have been reported by several workers (Gandhi *et al*., 1995; Sharma *et al*., 1997; Wink *et al*., 1997).

2.14 Growth studies: The significant correlation has been reported between different seed parameters (seed weight and seed length) and plant growth parameters (height and collar). The genotypes having the longest length and largest 100 seed weight registered the maximum collar diameter, plant height and germination index. The correlation between seed diameter and germination index has been observed to be non-significant. This may be due to the fact that some seeds may be empty despite of having big size. *Albizia lebbeck* with heavy seed recorded maximum germination as compared to light seed (Mutha *et al*., 2004). Likewise, the results on variation in germination parameters have been reported in *Acacia nilotica* (Neelu *et al*., 2000).

Depth of seed placement is important for phanerocotylon type of germination. *Physic nut* exhibits phanerocotylon type of germination therefore by depth of seed placement is important. Shallow (3-4 cm) seeds placement produced significant higher germination percentage, as well as all growth parameters. Quality index of seedling and chlorophyll contents could be noticed with over the deep (5-6 cm) placement of seeds (Gupta 1992; Arjunam *et al*., 1994 and Uma Rani *et al*., 1997).

In case of bold seeds more root and total length was recorded which explored greater soil volume for water and nutrients than normal seed which induced marked variation in quality and vigor of seedlings. Similar results were also reported by several workers (Chen 1999; Kokou *et al*., 2001 and Seiwa *et al*., 2002).
Variation in seed size of *Jatropha curcas* indicated a wide variation in its seed size and accordingly the seeds could be grouped into three size classes viz. small (13-15 mm), medium (15-17 mm) and large (17-19 mm) usually. Out of the total seed in a sample, maximum number of seeds (62%) was recorded in the medium size class, whereas small and large size classes were represented each by only 19%. Medium size class with 63% of the total sample weight was found to be the most prominent among the three size classes. All physical characters of seed differed significantly among various size classes. As the seed length increased, 100 seed weight and seed width also increased from small. Accession source of *Jatropha curcas* is being explored for its oil yield potential throughout the world. The source used in this study had mean annual rainfall range from 400 to 3350mm (Pathak *et al.*, 1981; Arunachalam *et al.*, 2003).

The 100 seed weight was found to have higher significant positive relationship with height 12 and 24 months field growth, stem girth (12 and 24 months field growth) and number of branches (12 and 24 month field growth). Growth traits viz. height, stem girth, 100 seed weight and field survival were found to have significant inter correlation with each other. It was found that heavier seeds have better seedling growth in the field Aslan, (1975). This result is in agreement with the findings of Oyin and Kassim (2006) who reported that the heights of *Celosia agentea* decreased as the concentration spent engine oil increased. Similar results were obtained with the control having the highest mean value of 5.59, 82.99 and 3.11 respectively, which also confirmed the reports of Oyin and Kassim (2006). Authors reported that number of leaves, leaf area and collar diameter of the plant decreased as spent engine oil concentration in the soil increased.
2.15 **Use of oil cake as a bio-fertilizer:** Seed cake contains curcin, a highly toxic protein similar to ricin in castor, making it unsuitable for animal feed. However it does have potential as a fertilizer or biogas production (Staubmann *et al.*, 1997; Gubitz *et al.*, 1999). Its availability in large quantities is used as a fuel for steam turbines to generate electricity. The defatted meal has been found to contain a high amount of protein in the range of 50-62% and the level of essential amino acids except lysine is higher than the FAO (Food and Agricultural Organization) reference protein (Makkar *et al.*, 1998).

Being rich in nitrogen, the seed cake is an excellent source of plant nutrient. In a green manure trial with rice in Nepal, the application of 10 tonnes of fresh *physic nut* biomass resulted in increase yield of many crops (Sherchan *et al.*, 1989). Another use of *Jatropha curcas* seed cake is as a straight fertilizer. Its properties were compared with those of other organic fertilizer with regard to nitrogen, phosphorus and potassium content which is shown in the *Jatropha curcas* seed cake and can be utilized as feed stock for biogas production (Karve, 2005; Visser and Adriaans, 2007).

The use of biogas slurry as a fertilizer is still in the early stages. Recently on solid state fermentation of *Jatropha curcas* seed cake showed that, it could be a good source of low cost production of industrial enzymes (Mahanta *et al.*, 2008).

2.16 **Use of oil cake as an insecticide:** The first and immediate possible solution to the problem is to reduce or, better still, eliminate the toxic element present in the seed cake from currently cultivated toxic *Jatropha curcas* crops, by treatments other than high temperature. Many approaches and patents have been proposed in order to detoxify the seed cake, either through removal of the PE by solvent washing treatments or by biotransformation (by means of fungus/yeast). In particular, after it was shown that, with the traditional oil refining process, including degumming,
deacidifications, bleaching and deorization, the PE content can only be reduced by approximately 50% (Haasw \textit{et al.}; 2000), many processes with the aim of eliminating the PE content from the \textit{Jatropha curcas} seed cake by chemical detoxification have been published.

A very interesting approach is biological detoxification for making \textit{Jatropha curcas} suitable for animal feeding. Experiments describe the complete degradation of PE by \textit{Pseudomonas aeruginosa} Pse A strain, during a solid-state fermentation of de-oiled \textit{Jatropha curcas} seed cake, in 9 days under optimized environmental condition (Joshi \textit{et al.}, 2011) and treatment of \textit{Jatropha curcas} seed cake by solid-state incubation with white rot fungi is able to promote the decrease of toxic element content to non-toxic levels (Debarris \textit{et al.}, 2011). Results obtained by a biotechnological approach and in particular by a cell culture technique, utilized to reduce the production of toxic compounds in new \textit{Jatropha curcas} plants (Wirasutisna \textit{et al.}, 2011). However, these detoxification treatments are non-specific and inefficient, not achieving a PE threshold that allows a direct use of detoxified seed cake in livestock feed and virtually all are not environmentally friendly processes, time consuming, difficult to implement, especially in developing countries and expensive, nullifying, at least partially, the effort to improve the economic sustainability for whole \textit{Jatropha curcas} bio-fuel chain. For these reason, if the detoxification process is currently the only suitable means to allow \textit{Jatropha curcas} seed cake to be used as animal feed, for the future it is necessary to identify more economical and environmentally sustainable approaches.

\textbf{2.17 Use of oil cake as a microbial growth promoter:} The fungal species isolated from the water source as linked with previous reports show that they are potentially pathogenic depending on the immune status of an individual using these
water sources. This is consistent with the study of (Dorothy, 2009 and Ahmed, 2011), who demonstrated the pathogenic nature of some fungal species from environmental sources. Most of these fungal sources have the ability of growing in variety of synthetic medium studied for their diagnostic purposes (Anjisha et al. 2012). For chemotherapeutic purposes some medicinal plants were tested *Azadirachta indica, Ocimum gratissimum, Jatropha curcas* and *Carica papaya*. The evolution of the antifungal activities of plant extract from these sources showed that the medicinal plants exert some antifungal properties against almost all the fungal isolates. Although at different level with most of the isolates moderately affected while the growth of only few of the isolates were totally inhibited by the plants isolates out of the three plant extracts used, extract from neem was found to be most active against the fungal isolates while the extract from *Carica papaya* was found to be least active. It can be concluded that the four plants used in this study are potential sources of active antifungal compounds that can be employed for therapeutic and sterilization purposes. The extract with further study can be optimized and purified for effective therapeutic purpose.

2.18 Comparison of normal field soil with the soil which is mixed with de-oiled cake powder of *Jatropha curcas* seeds:

Kumar *et al.* (2008) reported that a methanolic extract of *C. colocynthis* fruits collected in the area of Haryana in India contained, as polyphenols, 740 mg of gallic acid equivalent per 100 gm plant matter and, as flavonoids, 130 mg of catechin equivalent per 100 gm plant matter.

Phyto constituents were found to be disrobed (such as saponins, tannins flavonoids and alkaloids) in distinct parts of plant *C. colocynthis*, e.g. in leaves fruits, roots and seeds (Gill *et al.*, 2011). Studies conducted and documented the presence of
alkaloids, steroids, terpenoids, flavonoids, as well ascoumarins, glycosides in methanolic and hydromethanolic extracts of *C. colocynthis* seeds. According to Suman (2010) the roots of this plant were found to rich with alkaloids, flavonoids, terpenoids and glycosides, but no saponosides and anthraquinones. Minor differences could be noted related to local climate and soil composition.

The extract of *Ocimum sanctum* and *Azadirachta indica* inhibited the growth of *C. capsici* by 43% and 64% respectively (Sinha *et al.*, 2004). Shukla and Tripathi (1987) reported that the oil of *Pimpinella anisum* at 1000 mg/1 exhibited total lethality on *C. capsici* antifungal species.

### 2.19 Uses of Latex as a medicine:

The latex also showed a very good activity against *Candida albicans* and *Tricophyton sp*. The antimicrobial activities of the latex could be due to the presence of secondary metabolites such as tannins, flavonoids and saponins which have been confirmed to be present (Levens *et al.*, 1979). Flavonoids are phenolic in nature and inhibit the activity of enzymes of pathogens (IWU *et al.*, 1990; Pathak *et al.*, 1991).

The liquid latex has losses all its activity within a week while the dried latex maintains its activity up to fourteen week period. This indicates that hydrolysis play a major role in the degradation of the latex. The main target classes of hydrolytic reactions are amides, and esters (Florence and Attwood, 1981), suggesting that these compounds may be the main components of the latex.

The IR spectrum of EAE indicated the presence of aromatic phenolic compounds are generally behave as acids (Gisvold, 1977) with high antimicrobial activity remains in the unionized form which is more lipid soluble than the ionized form and, can gain more entry through cell membranes. Phenolic compounds are susceptible to attack by oxygen of the air and by oxidizing agents, which converts
them to the corresponding quinines (Gisvold, 1977). The degradation of the latex by hydrolysis and heat may suggest the main active components of the latex are phenolic in nature, undergoing hydrolysis and oxidation in the presence of unsuitable conditions.

Enzymatic activities have been also observed in the powdered sample as the latex has been found to contain some enzymes (Nath and Dutta, 1992, Auvin et al., 1977).

2.20 Uses of seed as a medicine: The seeds of *P. corylifolia* are used in indigenous medicine as laxative, aphrodisiac, antihemintic, diuretic and diaphoretic in febrile conditions. They have been specially recommended in the treatment of leucoderma, leprosy, psoriasis and inflammatory disease of the skin and are prescribed both for oral administration and for local external application in the form of a paste or ointment (Latha and Panikkar, 1999). A number of preparations made from the seeds have been tried in numerous cases of leucoderma and other skin diseases, oral administration of the powdered seed. Patients advised generally in curing nausea, vomiting, headache. The seed extracts inhibit the growth of *Staphylococcus citreus, S. aureus and S. albus* including strains resistant to penicillin. The seed possesses antihelmintic activity against earth worms (Wang et al., 1999).

The antibacterial potential of the seed extract of *P. corylifolia* has been demonstrated. However, none of earlier reports have been demonstrated the anti patency of *P. corylifolia* against phyto pathogenic fungi in general and biodeterioration causing fungi in particular (Chistawar et al. 1992; Haraguchi et al., 2000: Newton et al., 2002).
2.21 **Uses of stem and root bark:** Traditionally plant extracts are used as medicine. Practice continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). *Jatropha curcas* used in transitional folklore medicine has to cure various ailments in Africa, Asia, and Latin America (Bukill, 1994). Previous studies have been reported that plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm and joint rheumatism (Irvine, 1961; Oliver-Bever, 1986; Fagbenro-Beyiokoku, 1998). Anti-parasite activity of the sap (latex) and crushed leaves of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity (Matsuse *et al*., 1999).

Antimicrobial activities have been observed in many species of *Jatropha curcas* (Aiyelaagbe *et al*., 2000; Aiyelaagbe, 2001).

2.22 **Root bark as medicine:** Tritom which is used as an emulsifier does not influence the effectiveness of neem oil. Munakata (1977) described the repellent action of seed or leaf extract against the migratory locust; *Locusta migratory* and desert locust, *Schistocerca gregaria*.

*Jatropha curcas* roots are used against diseases caused by fungi and bacteria. Previous work (Dekker *et al*; 1986 Aiyelaagbe *et al*., 2000; Aiyelaabe, 2001) has shown that many *Jatropha curcas* species possess antimicrobial activities. Alkaloids and tannins are also present in the entire root, wood and bark extracts of *Jatropha curcas* (Rahila *et al*., 1994).

2.23 **Separation of microorganisms:** Warcup, (1950) method for isolation and identification of fungi and bacteria had been followed by several earlier workers. Many microorganisms that can degrade PHA have been reported. Hoang *et al*. (2007) isolated 135 actinomycetes from upstream and downstream regions of Touchien river in Taiwan which can degrade PHB, microbes which can degrade other poly (butylene succinate) and plates.
PHB films are mainly degraded by microbial activity. PHB films serve as more readily available carbon deficiency takes place causing it to be more rapidly disintegrated (Reddy et al., 2003; Dai, 1990; Kasuya et al., 1996). Degradation of PHB films takes place by surface erosion mechanisms. Bacteria attached to the porous area on the film surface secrete depolymerase to perform catalytic action on the polymer.

2.24 **Antifungal and antibacterial studies of oil:** The higher bioactivity of *Colletotrichum truncatum* EF10 was also observed in its extracts. All the isolates of *Colletotrichum truncatum* were found yielding oil, and fatty acid composition of oil isolated from EF10, was similar to *Jatropha curcas* seed oil. *Colletotrichum truncatum* isolate EF10 also recorded highest yield, while its methanol extract recorded very good antifungal activity. Endophytic association of *N. oryzae* is well documented and reported from crop plants, including maize (Saunders and Kohn 2008).

The result of minimum bacteria concentration of the extracts showed that with the exception of the antibacterial assays against *Staphylococcus aureus* and *E. coil*. MBC at a concentration of 500 mg/ml while the hexane extracts from 250-500 mg/ml in *S. aureus* was found more susceptible Gram negative bacteria such as *S. typhimurim*, *P. aeruginosa*, *E. coli* and *V. cholarae* methanol extract of neem *Azadirachta indica* oil at 1000 mg/I reduced the growth of *Curvularia lunata* by 26% (Govindachari et al., 1998).

2.25 **Antifungal and antibacterial studies of cake *Jatropha curcas***: Several plant extracts are known to possess nematotoxic properties (Sosamma and Jayasree, 2002). Reduction of nematode population may be attributed to the production of nematicidal substances like terihienyl, triterpenoid and other alkaloids by organic compounds (Nandal and Bhatti 1993; Trivedi et al., 1980). Rastogi and Mehrotra (1995) isolated two triterpene ester with biological activity from *Calotropis procera* leaves.
Plant leaf extracts of *Datura stramarium* and *Calotropis procera* were found to be highly significant in reducing radicle growth of the pathogen. The other extranets in order of superiority were *Azadirachta indica*, *Jatropha gossypifolia* Linn., *Lawsonia inermis*, *Eichhornia crassipe*, *Verbesena enceloides* and *Morus alba*. The aqueous extracts of these plants were found to affect the growth of the fungus. It is therefore encouraging to identify and characterize the active principle. Moreover, because of the water soluble nature of the toxic principal, it is ideal for development into herbal pesticides. The inhibitory effect of plant extract might be attributed to the presence of some antifungal toxicants.

Several authors have also reported fungicidal activity in wide variety of taxa. Ravichandar (1987) reported that the growth of *R. solani* was completely inhibited with the leaf extract of *Acacia nilotica*. Neem leaf extracts is known to reduce the viability of *R. solani* and mycelial growth considerably in vitro (Rao *et al.*, 1988; Grewal and Grewal, 1988), has mentioned differential fungicidal properties of leaf extracts of *Azadirachta indica*, *Chrysanthemum indicum* and *Tagetes erecta* against various weed moulds of mushroom by Sarkar *et al.* (1988) used leaf extracts of casuarina and water hyacinth for reducing the incidence of weed fungi.

The presence of antifungal compounds in higher plants is well recognized and considered valuable for plant disease control (Singh and Dwivedi, 1987). Various plants extracts have been evaluated for their antifungal property against different pathogens (Tripathi *et al.*, 2002).

### 2.26 Screening of leaf extract: Antibacterial, antifungal and percentage inhibition

of the methanol, ethanol and aqueous leaf extracts of *R. communis* have been evaluated by earlier researchers. The in vitro antimicrobial activity of different plant extracts was first step towards the development of new potential drug. Methanol leaf
extract of *R. communes* exhibited maximum antimicrobial activity and water extract showed minimum activity against four bacterial *S. Aureus, B. subtilis, P. aeruginosa* and *K. pneumoniae* and two fungal strains *A. fumigatus* and *A. flavus* (Chandrasekaran and Venkatesalu, 2004).

The activity of water extract is also reported by Ashafa *et al.* (2008) and Aiyegoro *et al.* (2008). Methanol leaf extract is more effective against pathogenic bacterial strains than ethanol or water extract (Naz *et al.*, 2011).

Fungi are major skin disease causing organisms. Many species of fungi are also found by, several worker as, plant pathogens. Antifungal activity of *Rhizoctonia communes* was studied. Methanol leaf extract exhibited about 59.5% and 56.3% fungal inhibition against *Aspergillus fumigatus*. The recent studies indicated that the antibacterial and antifungal activity vary with the different solvents of plant leaf material. *Rhizoctonia communes* is effective even at low concentration against bacterial and fungal pathogens.

### 2.27 Screening of stem bark:

The water extract of the plant indicated the antibacterial activity with inhibition zones ranging between 0 and 8 mm for different bacteria in general. The methanol extract was observed to show more activity than other extracts. This may be attributed to the presence of soluble phenolic and polyphenollic compounds (Kowalski and Kedzia, 2007).

The inhibitory effect of the extract of *Jatropha curcas* against pathogenic bacteria strain can introduce the plant as a potential candidate for several pathogens. Aqueous extracts of plants generally showed little or no antibacterial activities (Koduru *et al.*, 2006; Aliero *et al.*, 2006; Ashafa *et al.*, 2008; Aiyegoro *et al.*, 2008; Mujumdar *et al.*, 2001). The crude methanol extract from the root of *Jatropha curcas* exhibited anti-diarrhea activity in mice (Aiyelaagbe *et al.*, 2007). It has also been
reported earlier that the presence of some secondary metabolites in the root extract of *Jatropha curcas* inhibited some microorganisms isolated from sexually transmitted infections.

The susceptibility of some fungi to *Jatropha curcas* extract is significant as most of the fungi have recently been implicated in cases of immuno-compromised patient (Portillo *et al.*, 2001).

Generally the methanol extract had the highest activity against both bacterial and fungal isolates. This was followed by the ethanol extract and the least was observed in the water extract. The inhibitions of the growth of several bacterial and fungal species are an indication of the broad spectrum antimicrobial potential of *Jatropha curcas* which makes the plant a candidate for bioprospecting for antibiotic and antifungal drugs.

Herbs that have tannins as their main compounds are astringe it in natural and are used for treating intestinal disorder such as diarrhea or dysentery (Dharmananda, 2003). Activities against pathogens have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori *et al.*, 1994).

Alkaloids which are one of the largest groups of phyto-chemicals in plant have amazing effects on human and this has led to the development of powerful pain killer medications (Kam and Liew, 2002) which may be the effect of saponins on inflamed cells.

2.28 **Root bark extract:** The extracts showed strong antimicrobial activity against the organisms at a concentration of 200 mg. Alkaloids and tannins are also present in all the root wood reported in literature (Rahila *et al*. 1994). All the methanol extracts are richer in there metabolite than the hexane and ethyl acetate extracts which may be due to the higher polarity of methanol, hence its ability to extract more components.
It is noteworthy that the methanol extract of the root bark showed potent broad-spectrum activity against all the microorganisms except *Candida albicans*. It is even more active than Gentamycin, with respect to *E. coli*, *Klebsiella*, *Aerogenes* and *Pseudomonas aeruginosa* (Rahila *et al*., 1994).

*Jatropha curcas* roots are used for eczema, ringworm and gonorrhea and these diseases are caused by fungal and bacterial infections. Previous work (Dekker *et al*., 1986, Aiyellaabe *et al*., 2000; Aiyelaagbe 2001) has been shown that many *Jatropha curcas* species possess antimicrobial activity, but this is the first report of antimicrobial activity of the extracts of *Jatropha curcas* against bacteria and fungi STD microorganisms. The antimicrobial activity of these extract explains many uses of the plant in ethno-medicine.

2.29 De-oiled seed cake extract: All the extracts assayed indicated no activity to weak inhibitory activities against several fungi based on the antifungal activity of extracts from different plants parts viz. pericarp and mesocarp showed no antifungal activity against several fungi but seed extract at the concentration of 0.3 mg/l well showed antifungal activity. *Aspergillus* showed higher with inhibition this inhibition was low compared to Amphote ricain. Microorganisms showed sensitivities to different extracts of *P. macro carpa* fruit. These results might be due to the presence of phytochemical in seed such phorbol esters (Borris *et al*., 1988).

Antimicrobial activities observed in the studies might be due to the presence of flavonoid compounds. Extracts of various medicinal plants containing phenolic and favonoids have been previously reported to possess antimicrobial activity (Ayaz, Hayiriloglyu – Ayz, 2008; Rahman and Moon 2007; Vaquero *et al*., 2007).

Investigated the properties of gallic, caffeic, vanillic acid, rutin, and quercetin of different wine against pathogenic microorganisms. *E. coli* were the most sensitive.
bacterium and Flavobacterium sp. was resistant against several phenolic compounds. The flavonoid analysis revealed the presence of kaempferol, myricetin, naringin, quercetin and rutin in P. macrocarpa fruit. The presence of these compounds might contribute to antimicrobial activity of P. macrocarpa fruit.

Cushine and Lamba (2005) reported that kaempferol, myricetin, naringin, quercetin and rutin have antimicrobial activity against human pathogenic microorganisms with some mechanisms of action such as inhibition of nucleic function and energy metabolisms.

The antimicrobial activity of the extracts of P. macrocarpa fruit might be due to one of the mechanisms of action mentioned above antimicrobial activity from pericarp, monocarp and seed of P. macrocarpa fruit. Teffo et al., (2010) isolated four kaempferols from Dodonaea viscosa leaf extracts and pathogenic bacteria. The results showed the all isolated kaempferols could inhibit growth rate of Staphylococcus aureus, Enterococcus faecalis, E. coli and Pseudomonas aeruginosa. In addition Demotzos et al., (2001), investigated the antimicrobial activity of myricetion and its derivates and the result showed that they could inhibit Gram positive bacteria compared to Gram-negative. Furthermore (Li, et al., 2008; Bennett, 2007) mentioned that quercetin and naringin have antimicrobial activity.