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Isolation Of Exopolysaccharide Producing Microorganism)

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Abstract : In recent years, there has been an increasing demand for the isolation and identification of new microbial polysaccharides that can compete with traditional polymers because of their improved chemical and physical properties, higher flocculating and emulsifying activities, and Microbial polysaccharides have pesticidal activities. Over the past few decades, chemical pesticides were widely used in agricultural practices. However, the widespread and prolonged use of these chemical pesticides resulted in bio-magnifications of insecticides and insecticide resistance, which in turn resulted in the export of agricultural products. The main advantages of biopesticides are less harmful, designed to affect only one specific pest, very effective in very small quantities, degrade quickly and with very less pollution. Currently, emergence of drug resistant bacterial strains has put forward an immediate need for other alternatives of antibiotics which can be efficient in controlling the infectious conditions caused by these strains. EPSs from such bacteria can serve as better and may be the novel source to combat such infections. Therefore, the present study was aimed to isolate efficient EPS producing bacteria. Studied the extracellular polysaccharide producing bacteria *Xanthomonas* Isolate and identified by biochemical characterization to This work attempts to synthesize xanthan using infected part of lemon by batch fermentation method. And finally, the effect of larvicidal and antibacterial activity was checked.

Keywords:- polysaccharides, biomagnification, biopesticides, exopolysaccharides, fermentation.

INTRODUCTION

Introduction:-

Many microorganisms, yeast and fungi will manufacture carbohydrates. Though a lot of interest during this chemical compound is thanks to their role in infection or adhesion, a number of them have well-tried to be helpful industrial merchandise that vie with plant and protoctista carbohydrate likewise as artificial merchandise. whereas dextran was the primary microbic carbohydrate to be commercial and to receive approval for food use, many such polymers currently have a range of business uses. amazingly, a number of the carbohydrates fetch comparatively high costs. Only two, each microorganism polysaccharides, are area unit presently utilized within the food business, except in Japan where all such polymers are thought to be natural merchandise. If you browse the label on several factory-made foods on grocery shelves or on the sachets of dressing you receive in planes, you'll realize several contain Xanthan, a microorganism carbohydrate. This product is currently terribly widely utilized by the food industries of Europe and North America and is made within the US, Great Britain and France likewise as different countries.

Production of most microbial polysaccharides involves growth in stirred tank fermenters exploitation media with aldohexose or disaccharide because of the carbon and energy supply. Synthesis is commonly favored by high C:N ratios. owing to the high seriousness of the fermentation broths, economical combining and aeration area unit needed beside appreciable energy input. Fed-batch fermentation could also be desirable to the employment of high initial sugar concentrations. Once pasteurization of the broth, recovery by precipitation with iso-propanol is followed by drying and grinding to yield fine powder. Filtration or natural processes and different downstream processes augment the ultimate price.

Xanthan may be a product from the plant infective agent Xanthomonas citri. it's a plastic backbone on each second aldohexose residue of an oligosaccharide aspect chain. This uncommon structure confers physical properties to the chemical compound that is used in food and different industries. Xanthan is stable at each acid and alkalescent hydrogen ion concentration and forms pseudo plastic dispersion in water. comparatively low carbohydrate concentrations manufacture extremely viscous solutions compatible with several different ingredients in food and provide smart flavor unharness. Xanthan is additionally smart suspending and helpful agent for oil/water emulsion like sauce, owing to of these options and its inherent safety, xanthan received GRAS listing (Generally thought to be Safe) for food use within the US once its initial discovery within the Department of Agriculture laboratories in metropolis and its development by KELCO. Later, the carbohydrate received approval within the EU.

Most phytopathogenic microorganisms don't kind spores. Several of them are unit proof against desiccation and survive underneath dry conditions for over fifty years at traditional close temperature. this can be thanks to the protecting layer of the 'ooze' or the exudates made by the microorganism. This coating could act as a barrier against attack from bacteriophages, and conjointly helps in identification of applicable sites on the host plant for settlement of the microorganism.

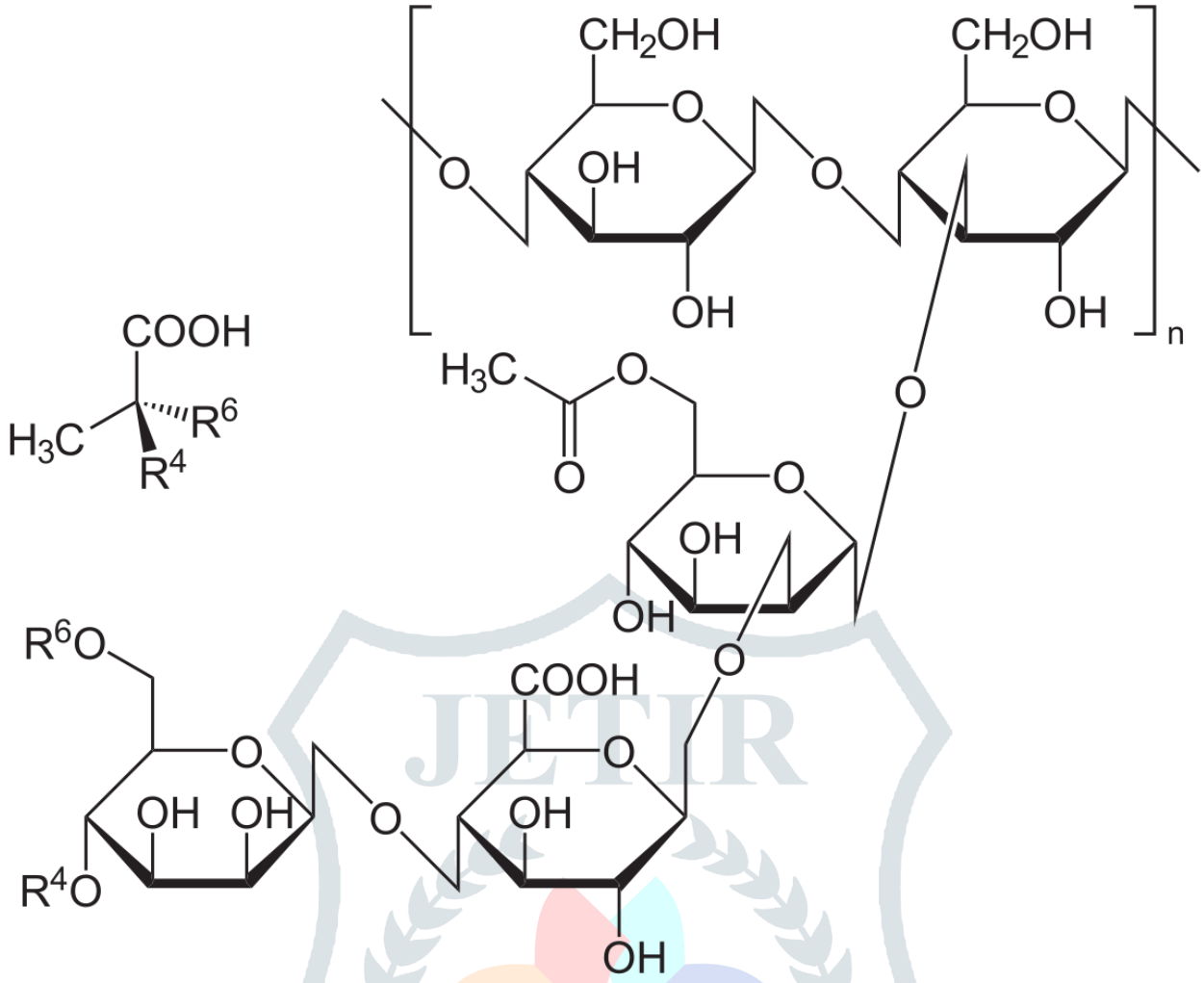
During this gift work, Xanthan gum is made by submerging aerobic fermentation by exploiting Xanthomonas spp. Isolated from citrus canker. At the top of the fermentation, the broth contains xanthan, microorganism cells, and lots of different chemicals. For ill the xanthan, the cells area unit typically removed 1st, either by natural process or filtration.

more purification could embrace precipitation exploitation, water-miscible non-solvents (iso-propanol, ethanol, and acetone), addition of sure salts, and hydrogen ion concentration changes. Once precipitation, the merchandise is automatically dewatered and dried.

Chemical structure or structural Backbone of xanthan gum

Xanthan gum is heteropolysaccharide with a primary structure consisting of perennial Penta macromolecule units fashioned by 2 aldohexose units, 2 mannose units, and one glucuronic acid unit, in within the molar quantitative relation of 2:8:2:0:2:0. Its main chain consists of a b-D-glucose unit connected at one and four positions. The formula of xanthan area unit (C35H49O29). The chemical structure of the most chain is the image of that of polysaccharide. the oligosaccharide aspect chain contains a D-glucuronic acid unit between 2 D-mannose units connected at the O-3 position of each different aldohexose residue within the main chain. Just about a simple fraction of the terminal D-mannose contains an acid residue connected via keto cluster to the 4and half dozen positions, with associated unknown distribution. The D-mannose unit connected to the most chain contains associate acetyl at position O-6. The presence of ethanoic acid associated with acid produces an anionic carbohydrate sort.

The oligosaccharide branches seem to be closely aligned with the chemical compound backbone. The ensuing stiff chain could exist as one, double, or triple helix, that interacts with different chemical compound molecules to create a posh. The materia medica and safety properties of xanthan gum for food and pharmaceutical applications are extensively researched. xanthan is non-toxic and doesn't inhibit growth. it's non sensitizing and doesn't cause skin or eye irritation. On this basis, xanthan has been approved by the us Food and Drug Administration (FDA) to be used as an artificial additive with non specific amount limitations. In 1980, the ecu Community other the food emulsifier/stabilizer list as item E-415. Xanthan gum has been employed in a large style of food for variety of vital reasons, together with emulsion, stabilization, temperature stability, compatibility with food ingredients and its pseudo plastic natural philosophy properties. owing to its properties in thickening binary compound solutions, as dispersing agent, and stabilizer of emulsion and suspensions, xanthan gum is issued in pharmaceutical formulations, cosmetics, and agricultural merchandise. it's employed in textile printing paste, ceramic glazes, suspension explosive, formulations and rust removers. High seriousness of solutions and water solubility of chemical compound have created vital applications for xanthan within the fossil fuel business wherever it's normally employed in drilling fluids and in increased oil recovery processes.



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Biosynthesis of Xanthan gum

Xanthan is complex microbial exopolysaccharides industrially produced from glucose via fermentation by the plant pathogenic bacterium, Xanthomonas citri.The synthesis of Xanthan gum is believed to be similar to exopolysaccharide synthesis by other Gram-negative bacteria. The synthetic pathway can be divided into 3 parts:

• Uptake of simple sugar and conversion to nucleotide derivatives.

• Assembly of Pentasaccharide subunits attached to an iso prenyl pyrophosphate carrier.

• Polymerization of Pentasaccharide repeats unit and their secretions

Xanthan is produced by bacterium Xanthomonas citri isolated originally from lemon in which it causes citrus canker disease. Xanthomonas species are Gram negative bacteria taxonomically placed in the pseudomonas family. Accordingly, to the genetic basis of classification, the average genome size of Xanthomonas is 2.5 x 109Da. The G+C percent content of the DNA ranges from 63-71%. The cells have rod-like form, slightly curved with rounded ends, measuring 0.2-0.6 by 0.8-2.9 micrometer. They occur mostly alone or in pairs, but chains and filaments are also observed. X.citri forms smooth yellow mucoid colonies on solid media, and cells are surrounded by xanthan gum (EPS).

The chemical structure of the yellow pigment was determined to be mono- or dibromo aryl polyene and is probably bound to the cellular membrane, genetic instabilities that affect quality and yield of xanthan than have been observed in Xanthomonas cultivation Genetic mutations are associated with the formation of L. (large) and S (Small) colony types. Whereas L-type colonies produce high xanthan yield with the desired rheological characteristics, the S-type colonies give low product yield with poor quality.

The natural function of xanthan is not fully recognized. But there is strong evidence indicating that the polysaccharide layer surrounding the microbial cell protects the microorganisms from various environmental factors. The water retaining capacity of xanthan, for example, provides the microorganism with a protective layer under dry environmental conditions, thus providing some degree of desiccation resistance that reveal that xanthan biosynthesis is induced under stress conditions.

In Xanthomonas citri, the Entner-Doudoroff pathway in conjunction with the TCA pathway is the predominant mechanism for glucose catabolism A small portion of glucose is routed via the pentose phosphate pathway for glucose uptake; two discrete systems seem to exist. Biosynthesis of the xanthan as in most polysaccharides-producing bacteria, utilizes various activated carbohydrate donors to form the polymer as an acceptor molecule. The oligosaccharide repeated units of xanthan are constructed

by the sequential addition of monosaccharides from sugar nucleotide diphosphate to an isoprenoid lipid acceptor molecule. At the same time, acyl substitutes are added from appropriate activated donors as shown in (fig1), the xanthan backbone is formed by the successive addition of D glucose -1-phosphate and D-glucose from two moles of UDP-D-glucose. Thereafter, D mannose and D-glucuronic acid are added from GDP-mannose and UDP-glucuronic acid, respectively O-Acetyl groups are transferred from acetyl Coa to the internal mannose residues and pyruvate from phosphoenolpyruvate is added to the terminal mannose.

This pattern of reaction was elegantly demonstrated, using pre-metallized cells and radioactively labeled precursors. Further it was also shown that each of these steps as just described, requires specific substrates and a specific enzyme for completion. If either the substrate or the enzyme is absent the step is blocked.

It has been suggested that the construction of the exopolysaccharides follows a "tail -to-head" polymerization. After the Pentasaccharide repeated units are formed. Oligomers are formed by transfer to other lipid intermediates, gradually increasing the size of the carbohydrate chain. As noticed by Sutherland, oligomer construction normally involves the addition of the longer oligosaccharide sequence to the non-reducing terminus of a single repeat unit attached to the isoprenoid lipid diphosphate The inactive lipid carrier is dephosphorylated to yield iso-prenyl phosphate which can then re-enter the biosynthetic sequence.

Although the structure of the repeating units is determined by the sequential transfer of the different monosaccharide and acyl group from their respective donors by highly specific sugar transferees, the polymerase enzyme responsible for polymerization of the Pentasaccharide into macromolecule of M-106 has been shown to be less specific The final stages of exopolysaccharide segregation from the cytoplasmic membrane passing across the periplasm and the outer membrane and finally excreted into the extracellular environment are much less well specified than the previous biosynthetic events. This mechanism must exist in all polysaccharide producing bacteria for releasing polymer from the isoprenoid lipid prior to transport to its final destination. The process obviously requires an energy source and may be analogous to the export of lipo-polysaccharide to the outer membrane in which ATP is energy supplier.

Market size of xanthan gum

Xanthan gum currently dominates the microbial gum market, which is growing at between 6 & 7 % per year. It has been suggested that global production exceeds 50000 tons per year. The worldwide xanthan market is valued between $ 600 to $ 800 million per year. The European Union market accounted for about $ 158 million in 1994 and the U.S market was reported to be about $120 million in 1995. (Table 1.1) shows the US market size and forecast of Xanthan gum consumption by major end users. Food accounts for about 70% of total consumption; the remainder is spread among petroleum production, cosmetics, and other uses. Overall growth is forecasted at about 7.0 %, yearly, with food applications providing most of the impetus at 8.3 % annually. Consumption of xanthan in petroleum is forecasted at a very modest rate (2%), which is due mainly to recovery operations rather than oil well drilling. Because of the unique properties, the application of the xanthan in cosmetics has been growing successfully at 5.9% per year. It should be noted that use in textile production is very nominal because the textile industry in the United States is conservative and only growing with the national economy.

Analysis of (Table 1) shows that xanthan is positioned well in the US market. Further analysis reveals that xanthan gum represents the fastest growing segment of the

Table- Market analysis and Forecast Xanthan consumption in the United States

End use	1993($)	1995($M)	1998($M)	AAGR(%) 1993-1998
Food	74.4	89.5	111.0	8.3
Petroleum	16.5	17.5	18.2	2.0
Cosmetics	9.0	10.2	12.0	5.9
Textile	1.4	1.4	1.4	00
Others	1.1	1.1	1.7	8.5
Total	**102.4**	**119.1**	**144.3**	**7.1**

Source Business communication company.SM Millions of dollars(M), AAGR (Annual Average Growth Rate)

Average Growth Rate polysaccharide industry. Most growth is in Food where demand for natural gums is falling as a result of market share erosion by xanthan. The high growth rate of the xanthan market may explain not only why the major producers have all expanded in recent years, but also why Archer Daniel Midland co has decided to enter the xanthan market with an estimated 10000 tons of annual capacity Between 1992 and 1994, the global xanthan production capacity doubled in size. Recently, NutraSweet Kelco Co. The world's leading producer of xanthan has announced further expansion. In fact, an overcapacity has been created in the xanthan market, which may have two important consequences: 1) An accelerated penetration of xanthan into new markets and 2) Stiff competition for new polysaccharides. Success may be limited only to those polymers that have a clear advantage in performance or customer perception. Less expensive polymers that donors perform better may be disadvantaged due

to the high cost of incorporating new gum into the end product. An estimate from 1982, suggests that the testing required by the US Food and Drug Administration to gain food clearance approval for a new additive can cost about $2 million and may take up to 8 years. According to the market forecaster, the two main factors shaping the xanthan market in the next century are growing xanthan demand in developing countries and worldwide energy demand, which will stimulate EOR operations as reserves decline. Indeed, the larger strategic value of xanthan still remains in EOR operations, whose worldwide production at the beginning of 1996 was estimated to be 2.2 million barrels/day. The large economic value of xanthan in the EOR market can be assessed, as suggested by Moses, by looking at the amount of oil that remains in the earth crust (table) Thus, it becomes clear that the EOR markets are very large indeed. They are, however, low price markets that could be developed only through the use of much improved technologies with low production costs. To improve the competitive advantage of xanthan in EOR, more efficient fermentation processes and better control of the polymer quality are required.

Table-Approximate Value of OIL not Economically recoverable by present technology

Source Region	Estimated value of oil unrecoverable by present technologies
Asia pacific	0.6
Western Europe	0.8
Middle East	12.4
Africa	1.9
Western Hemisphere	4.1
Former East Block States	2.9
Total for whole world	22.7

Relatively high xanthan gum costs, some technical drawbacks and the relatively low oil price have prevented market entry in recent years xanthan usage in EOR is typically 0.4% w/w (About 0.5 kg /barrels of oil), which represents about 25% of the estimated equilibrium oil price ($20 /barrel) a value that is evidently too high for the oil industry. A recent survey of the EOR shows that polymer flooding is achieving commercial success. In this operation up to 35% of original oil in place has been recovered in the Junger basin (China). Polymer flooding is being used in several projects in France, Germany, China, India and Romania. For example, polymer flooding accounts for about 50% of EOR operations in China. Even when it is not exactly clear what % percent of xanthan gum is responsible for, the fact that the largest xanthan gum production plant has been brought on-stream for oil applications is an indication that xanthan use in EOR is not far away Analysts in marketing research are convinced that the use of xanthan as well as other microbial gun in EOR will become prevalent as increasing worldwide energy demand results in rising oil prices.

Properties of xanthan gum:-

One of the remarkable properties of the xanthan is its capability of producing a large increase in viscosity of a liquid by adding a very small quantity of gum of the order of 1% In most of the foods, it is used at 0.5% or as low as 0.005 % The viscosity of xanthan gum solution decreases with higher shear rates, this is called "Pseudo plasticity'. Food needs high viscosity at low shear rates to be stable but when consumed, they cannot seem to be thick and heavy in the mouth, (fairly high rates), but it will have good stabilization properties. Xanthan is highly soluble in both cold and hot water, and this behavior is related with the polyelectrolyte nature of the xanthan molecule. Xanthan solutions are highly viscous even at lower polymer concentration. These properties are useful in many industrial applications, especially in the food industry where xanthan is used as a thickener and also as stabilizer of suspensions and emulsions.

The thickening ability of the Xanthan solutions is related to viscosity with a high viscosity resistance flow. Xanthan solution is pseudo plastic, or shears thinning, and the viscosity decreases with increasing shear rates. The viscosity also depends on temperature (both dissolution and measurement temperature), the biopolymer concentration, concentration of the salt, and pH (5).

The properties of the xanthan gum are explained in brief below:

● Xanthan is a white to cream colored free flowing powder soluble in both hot and cold water, but insoluble in most organic solvents. Even at low concentration with other polysaccharide solutions. This property makes it a very effective thickener and stabilizer.

● Xanthan solutions are highly pseudo plastic but not thixo tropic i.e., even after high shear rates the initial viscosity is rebuilt instantaneously

● Xanthan is more pseudo plastic than most other hydrocolloids. This pseudo plasticity enhances sensory qualities (flavor release, mouth feel) in final products, cases processing (mixing and pumping) and guarantees good pour ability.

● Xanthan gum solutions are very resistant to pH variation, ie, they are very stable in both alkaline and acidic conditions.

● The thermal stability of the xanthan gum is usually superior to most other water-soluble polysaccharide.

● The viscosity of xanthan gum solution is completely recovered after heat treatment steps during food processing eg. Sterilization. The rheological properties of the final products thus remain stable, irrespective of being kept in the refrigerator, stored at room temperature or heated. Xanthan gum also improves the thaw/freeze stability of frozen foods.

● Xanthan is tasteless and does not affect the taste of other food ingredients. The caloric value of xanthan gum is very low (0.6 Kcal/g).

● Xanthan is compatible with most food, cosmetics and pharmaceutical ingredients.

● Xanthan gum has an excellent stability in the presence of acids.

● Xanthan solutions have unusually good comp ability and stability in the presence of the most salts, the addition of electrolytes such as Na and KCL, increases viscosity 7 stability.

Table-Typical properties of commercial xanthan gum

Sr No.	Property	Value
1	Physical status	Dry, cream-colored powder
2	Moisture (%)	8-15
3	Ash (%)	7-12
4	Nitrogen (%)	0.3-1.0
5	Acetate content (%)	1.9-6.0
6	Pyruvate content (%)	1.0-5.7
7	Monovalent salts (%)	3.6-14.3
8	Divalent salts (%)	0.085-0.17
9	Viscosity(cP)	13-15

Applications:-

The following is a summary of the many applications for xanthan gum or blends of xanthan Gum And galactomannans and their related functions and benefits.

No	Food applications	Usage (%)	Functions
I	**Salad dressing**	0.1-0.5	Provides easy pourability and good cling; suspends spices
1.	Bakery products	0.05-0.3	Binds water; improve texture
2.	Beverages	0.05-0.2	Enhances mouth feel; suspends fruit pulp
3.	Instant products	0.05-0.2	Contributes body; quick viscosity build up in cold. And hot water
4	Prepared foods	0.1-0.3	Stabilizers; avoid syneresis
5	Frozen food	0.05-0.2	Provides good freeze/thaw stability; contributes good texture
6	Dairy products	0.05-0.2	Inhibits syneresis; stabilize emulsion
7	Soaps; sauce and gravies	0.05-0.5	Gives good temperature stability
8	Topping	0.05-0.3	Stabilizes foam and emulsion
9	Meat products	0.2-0.5	Binds water; inhibit syneresis
10	Low calories products	0.1-0.5	Improves texture; stabilizes
II.	**Personal care applications**	**Usage (%)**	**Function**
1.	Tooth pastes	0.7-1	Provides easy pump ability and gives good stand on brush
2.	Creams and lotions	0.2-0.5	Stabilizes emulsion; gives creamy consistency
3	Shampoos	0.2-0.5	Controls rheology; suspends insoluble
III	**Industrial applications**	**Usage (%)**	**Function**
1	Agricultural chemicals	0.1-0.3	Suspends active ingredients; controls drift and cling
2	Cleaners	0.2-0.7	Provides good pH stability; extent contact time
3	Polishes	0.2-0.7	Suspend abrasive components
4	Water based paints	0.1-0.3	Controls rheology; stabilizes pigment
5	Textile and carpet printing	0.2-0.5	Control color migration
6	Adhesives	0.1-0.3	Control rheology and penetrations

7	Paper industry	0.1-0.2	Act as suspension aids and control rheology
8	Ceramic glazes	0.3-0.5	Suspends solid effectively
9	Oil drilling	0.1-0.4	Provides good stability against salt, temperature and shear
10	Enhance oil recovery	0.05-0.2	Functions as mobility control agent
IV	**Animal feed**	**Usage (%)**	**Function**
1	Liquid milk replacer	0.05-0.2	Stabilizes water insoluble ingredients
2	Pet food	0.1-0.4	Prevents syneresis
V	**Pharmaceuticals**	**Usage (%)**	**Function**
1	Suspension and emulsion	0.1-0.5	Provides excellent stability and good flow
2	Tablets	1.0-3.0	Retard drug release
3	Lozenges	0.3-1.0	Prolongs contact time of active ingredients

Basic structure of production process

The design of a xanthan production method depends on the economic needs to realize optimum performance with regards to productivity, product concentration, and yield, and at a similar time, to satisfy the required product quality and application specifications. However, this is often not an easy task as a result of, throughout the assembly method, the body will increase drastically so product concentration is sometimes low and product quality additionally varies. A compromise between the business targets and therefore the limitations inherent to the method should be reached at the commercial level by establishing, and therefore the instrumentation style and operation. Xanthan is made commercially in an exceedingly typical batch method exploitation of automatically agitated vessels and a culture of X. citri in an exceedingly appropriate fermentation medium. It's an associate degree aerobic method that runs for about a hundred hrs. below the subsequent operation conditions: 28-30°C, pH -7, aeration rate beyond zero.3 vm. And specific power input for agitation beyond one kW/m3.A product yield approaches fifty which there's typical within the normal method. The inoculums preparation includes many stages requiring completely different sets of reactors starting from ten L for initial seed culture up to 100m three for the assembly functions, the necessity volume being typically enlarged by an element of ten. below N-limitations, cells enter the stationary section. Xanthan is synthesized in each expansion and therefore the stationary phases, reaching the ultimate concentration of concerning twenty gL-1 xanthan biogenesis ceases as aldohexose is totally consumed. The consistency issue as a parameter of the physical science properties of xanthan will increase from fifteen mPa Sn to thirty,000 mPa Sn the pO2 born over the initial periods to a minimum worth corresponding in time to peak gas uptake rates throughout the expansion section. Therefore, each OTR and p02 decrease unceasingly toward the top of the fermentation, reflecting the impact of accelerating both bodies on the gas transfer rate. Once industrial grade xanthan is needed, the post fermentation starts with the sterilization of the fermentation broth to kill the microorganism cells. As a result of the bacteria genus being sensitive and non-spore formers, complete sterilization will without delay be accomplished but caution ought to be soft on the temperature of the transition point (Tm) for xanthan so as to avoid thermal degradation of the compound. The atomic number 69 for xanthan is concerned with 100°C however it varies with the entire ionic strength and therefore the magnitude relation of the contents of pyruvate and acetate teams.

Downstream processing of xanthan gum

Recovery of xanthan from the fermentation broth is mostly troublesome and high-ticket. the ultimate fermentation broth contains 10-30 g/L xanthan. 1-10 g/L cells, and 3-10 g/l residual nutrients, and broth is extremely viscous and troublesome to handle. A high body complicates removal from the broth additionally, combining of the processed broth with recovery chemical agent is power intensive due to the body For processing; the broth is sometimes diluted at stage of the method.

The main steps of the recovery method are deactivation and removal (or lysis) of the microorganism cells, precipitation of the compound, dewatering, drying and edge process should be dodged degrading the biopolymer the ultimate product is sometimes the dry powder or the targeted solutions. varied ways are developed to deactivate, lyse or take away cells from the broth. Treatment chemically (eg: alkali, salt, enzymes) by mechanical means that and thermal treatments are used. Chemical treatments at elevated pH scale will cause de-pyruvylation of the merchandise once enzymes are used, they need to be off from the medium and this adds to prices. Usually, the fermentation broth is pasteurized or sterilized to kill the cells. These thermal treatments additionally enhance xanthan removal from the cells. sterilization of fermentation broth at the upper temperature usually causes thermal degradation of the microorganism exopolysaccharides, once the broth is treated below correct conditions (80- 130°C,10-20 min, and pH 6.3-6.9) increased xanthan dissolution happens while not thermal degradation and disruption of the cells is

determined. The augmented temperature additionally reduces the body of the broth to ease removal of the insoluble by natural action or filtration. For extremely viscous xanthan broths, body reduction should proceed literation body is reduced strive dilution or heating the fermentation broth is sometimes diluted in water, alcohol or mixtures of alcohol and salts in quantities below those required for xanthan precipitation The diluted/heated broth is filtered to get rid of the solids. Filtration in presence of alcohol.

Xanthan in answer is viewed as a hydrophilic mixture forming a real answer in water. Precipitation of compound is achieved by decreasing the solubility the dissolved mixture exploitation ways like addition of salts, water, compatible non-solvents, and concentration by evaporation Recovery choices that are studied embody precipitation with organic solvents like fermentation alcohol and IPA, the utilization of mixtures of salts and alcohol and precipitation with power or powerfulness salts. Also, the utilization of ultra-filtration has bec rumored. the foremost common technique used for primary isolation and purification of the polysaccharides is precipitation exploitation of water compatible non solvents like alcohols. The lower alcohols (methanol, ethanol, IPA) and dimethyl ketone, that ar non-solvents for the polysaccharides, is other to fermentation broth not solely to decrease the solubility till section separation happens, however additionally to scrub out impurities like coloured elements, salts and cells. the number required depends on the character of the chemical agent. Total precipitation does not occur with monovalent salts such as sodium chloride.

The addition of a non-reagent promotes precipitation not by decreasing the water affinity of the polymer, but also by enhancing the binding of the cations, which are present. Thus, xanthan precipitates with lesser amounts of reagents when alcohol and salts are used in combinations. When xanthan is precipitated using the combination of salts and IPA, the quantity of alcohol needed is lower than if only IPA was added. Alcohol volume is reduced when monovalent salts are used but volume reduction is greater with divalent salts. However, the use of divalent cations leads to less soluble xanthan salt as the final product. The polymer concentration in solution also influences the volume of the precipitating agent needed. When the polymer concentration in solution is increased, a smaller quantity of alcohol is needed for precipitating the biopolymer. Once the polymer is obtained as a wet precipitate, it is dried, milled and packed. The precipitate is dried in a batch or continuous dryer, under vacuum or with forced circulation of an inert gas. This prevents combustion of the organic solvent in the precipitate. Most commercial xanthan has a final moisture content of about 10 %. After drying, the polymer can be milled to a predetermined mesh size to control dispensability and dissolution rates. Some commercial xanthan gums are differentiated by mesh size. Care must be taken in milling, so that excessive heat does not degrade or discolor the product Finally, the packing used must be water proof because xanthan is hygroscopic and subject to hydrolytic degradation.

Isolation of *Xanthomonas citri*:-

Collection of samples

Three different infected bacterial blight samples of pomegranate, bacterial spot sample of capsicum and bacterial spot samples of lemon were collected and stored at 4^{o} C until used.

Requirement for surface sterilization

- $HgCl_2$ solution-0.01%
- Alcohol-95% (95 ml alcohol+100ml water)

Method

The area was surface sterilized using 0.01% $HgCl_2$ solution for 30 seconds and washed with sterile water 4-5 times. With a sterile blade, the infected area was transferred into watch glass containing a few drops of saline. It was tested with the help of sterile forceps and blades. This preparation was then added to sterile Yeast Malt broth medium and kept on a rotary shaker at room temperature for 2 days, this process was required for enrichment of the organism. After 2 days a loopful of enriched broth was streaked on sterile Yeast dextrose carbonate agar medium. Incubated at room temperature for 2 days then the colony characteristics were noted.

Requirements for enrichment of organisms

- Yeast malt broth
- Flasks
- Bacterial suspension

Composition of yeast malt broth

- Glucose-20g
- Yeast extract-3g
- Malt extract-3g
- Peptone-5g
- Water -1000ml

Isolation of microorganisms-

Streak plate method was used and streaked on YDCA agar plate

Composition of Yeast Dextrose Calcium Carbonate Agar:-

- Dextrose-20g
- Yeast extract-10g
- Calcium carbonate-20g
- Agar-20g
- Distilled water- 1 liter

Observations:-

Different bacteria were isolated on the basis of colony characteristics observed from the YDCA media and biochemical test.

Table- Colony characteristics of isolated organisms.

Characters	Xanthomonas Isolate L-1	Xanthomonas Isolate P-1	Xanthomonas C-1
Size	2mm	4mm	2mm
Shape	Circular	Circular	Circular
Color	Yellow	Cream	Peach
Margin	Entire	Entire	Irregular
Elevation	Raised	Raised	Raised
Opacity	Opaque	Opaque	Opaque
Consistency	Mucoid	Mucoid	Mucoid
Gram staining	Gram negative	Gram negative	Gram negative
Capsule staining	Positive	Positive	Positive
Motility	Motile	Motile	Motile

Gram staining

Materials for gram staining of isolated microorganisms

- A glass slides
- Crystal violet
- Gram's Iodine
- 95% ethanol
- Safranin
- Blotting paper

Biochemical tests:-

Capsule staining

The main purpose of capsule stain is to distinguish capsular material from the bacterial cell. A capsule is a gelatinous outer layer secreted by bacterial cells that surrounds and adheres to the cell wall. Most capsules are composed of polysaccharides, but some are composed of polypeptides. The capsule differs from the slime layer that most bacterial cells produce in that it is a thick, detectable, discrete layer outside the cell wall. The capsule stain employs an acidic stain and a basic stain to detect capsule production.

Requirements

- Clean grease free slide
- Nichrome wire loop
- 24 hr. old culture of capsulated bacteria
- 1% Congo Red Solution
- Maneval's Stain

IMViC Tests

The IMViC Tests were designed to differentiate gram negative organisms on the basis of their biochemical properties and enzymatic reaction in the presence of enzyme substrate.

Table3.2 Biochemical characteristics of *Xanthomonas citri*

Test	*Xanthomonas citri*
Catalase test	+
Oxidase test	-
Indole production test	-
Methyl red test	+
Voges Proskauer test	+
Simon Citrate test	-

Results

Isolation

Different bacteria were isolated from lemon, pomegranate and capsicum and characterization was done.

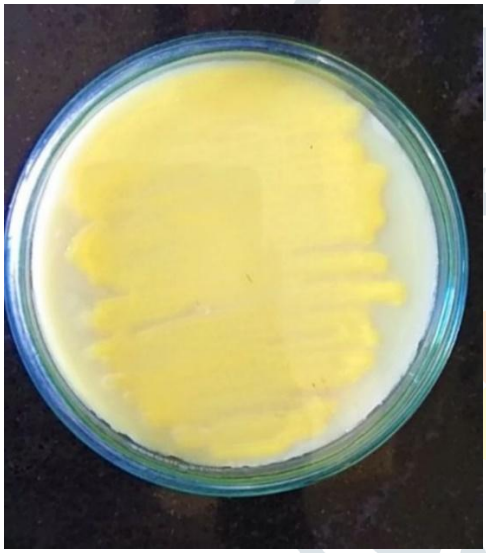

Fig.1 Isolation of L1

Fig.2 Isolation of C1

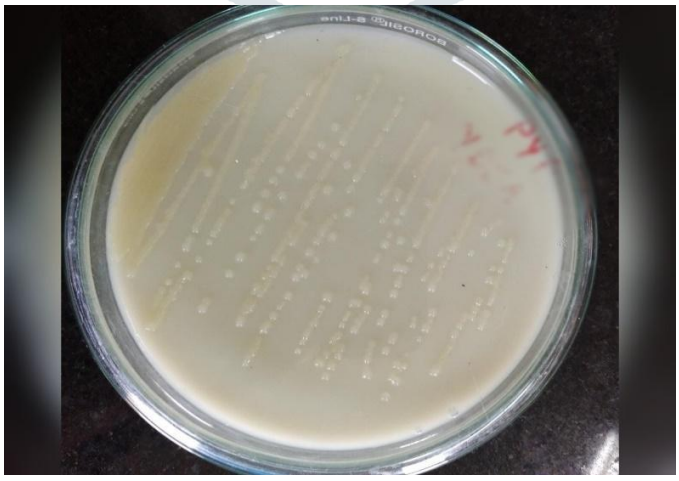

Fig.3 Isolation of P1

3.1Population and Sample

KSE-100 index is an index of 100 companies selected from 580 companies on the basis of sector leading and market capitalization. It represents almost 80% weight of the total market capitalization of KSE. It reflects different sector company's performance and productivity. It is the performance indicator or benchmark of all listed companies of KSE. So it can be regarded as universe of the study.Non-financial firms listed at KSE-100 Index (74 companies according to the page of KSE visited on 20.5.2015) are treated as universe of the study and the study have selected sample from these companies.

The study comprised of non-financial companies listed at KSE-100 Index and 30 actively traded companies are selected on the bases of market capitalization.And 2015 is taken as base year for KSE-100 index.

3.2 Data and Sources of Data

For this study secondary data has been collected. From the website of KSE the monthly stock prices for the sample firms are obtained from Jan 2010 to Dec 2014. And from the website of SBP the data for the macroeconomic variables are collected for the period of five years. The time series monthly data is collected on stock prices for sample firmsand relative macroeconomic variables for the period of 5 years. The data collection period is ranging from January 2010 to Dec 2014. Monthly prices of KSE -100 Index is taken from yahoo finance.

3.3 Theoretical framework

Variables of the study contains dependent and independent variable. The study used pre-specified method for the selection ofvariables. The study used the Stock returns are as dependent variable. From the share price of the firm the Stock returns are calculated. Rate of a stock salable at stock market is known as stock price.

Systematic risk is the only independent variable for the CAPM and inflation, interest rate, oil prices and exchange rate are the independent variables for APT model.

Consumer Price Index (CPI) is used as a proxy in this study for inflation rate. CPI is a wide basic measure to computeusualvariation in prices of goods and services throughout a particular time period. It is assumed that arise in inflation is inversely associated to security prices because Inflation is at lastturned into nominal interest rate andchange in nominal interest rates caused change in discount rate so discount rate increase due to increase in inflation rate and increase in discount rateleads todecreasethe cash flow's present value (Jecheche, 2010). The purchasing power of money decreased due to inflation, and due to which the investors demand high rate of return, and the prices decreased with increase in required rate of return (Iqbal et al, 2010).

I. RESEARCH METHODOLOGY

The methodology section outline the plan and method that how the study is conducted. This includes Universe of the study, sample of the study,Data and Sources of Data, study's variables and analytical framework. The detailsare as follows;

3.1Population and Sample

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Exchange rate is a rate at which one currency exchanged with another currency. Nominal effective exchange rate (Pak Rupee/U.S.D) is taken in this study.This is assumed that decrease in the home currency is inverselyassociated to share prices (Jecheche,2010). Pan et al. (2007) studied exchange rate and its dynamic relationship with share prices in seven East Asian Countries and concludethat relationshipof exchange rate and share prices varies across economies of different countries. So there may be both possibility of either exchange rate directly or inverselyrelated with stock prices.Oil prices are positively related with share prices if oil prices increase stock prices also increase (Iqbal et al, 1012).Ataullah (2001) suggested that oil prices cause positive change in the movement of stock prices. The oil price has no significant effect on stock prices (Dash & Rishika, 2011).Six month T-bills rate is used as proxy of interest rate. As investors arevery sensitive about profit and where the signals turn into red they definitely sell the shares. And this sensitivity of the investors towards profit effects the relationship of the stock prices and interest rate, so the more volatility will be there in the market if the behaviors of the investors are more sensitive. Plethora (2002)has tested interest rate sensitivity to stock market returns, and concluded an inverse relationship between interest rate and stock returns. Nguyen (2010) studies Thailand market and found thatInterest rate has aninverse relationship with stock prices.

KSE-100 index is used as proxy of market risk. KSE-100 index contains top 100 firms which are selected on the bases of their market capitalization. Beta is the measure of systematic risk and has alinear relationship with return (Horn, 1993). High risk is associated with high return (Basu, 1977, Reiganum, 1981 and Gibbons, 1982). Fama and MacBeth (1973) suggested the existence of a significant linear positive relation between realized return and systematic risk as measured by β. But on the other side some empirical results showed that high risk is not associated with high return (Michailidis et al. 2006, Hanif, 2009). Mollah and Jamil (2003) suggested thatrisk-return relationship is notlinear perhaps due to high volatility.

3.4Statistical tools and econometric models

This section elaborates the proper statistical/econometric/financial models which are being used to forward the study from data towards inferences. The detail of methodology is given as follows.

3.4.1 Descriptive Statistics

Descriptive Statics has been used to find the maximum, minimum, standard deviation, mean and normally distribution of the data of all the variables of the study. Normal distribution of data shows the sensitivity of the variables towards the periodic changes and speculation. When the data is not normally distributed it means that the data is sensitive towards periodic changes and speculations which create the chances of arbitrage and the investors have the chance to earn above the normal profit. But the assumption of the APT is that there should not be arbitrage in the market and the investors can earn only normal profit. Jarque bera test is used to test the normality of data.

3.4.2 Fama-Mcbeth two pass regression

After the test statistics the methodology is following the next step in order to test the asset pricing models. When testing asset pricing models related to risk premium on asset to their betas, the primary question of interest is whether the beta risk of particular factor is priced. Fama and McBeth(1973)develop a two pass methodology in which the beta of each asset with respect to a factor is estimated in a first pass time series regression and estimated betas are then used in second pass cross sectional regression to estimate the risk premium of the factor. According to Blum (1968) testing two-parameter models immediately presents an unavoidable errors-in-the variables problem.It is important to note that portfolios (rather than individual assets) are used for the reason of making the analysis statistically feasible.Fama McBeth regression is used to attenuate the problem of errors-in-variables (EIV) for two parameter models (Campbell, Lo and MacKinlay, 1997).If the errors are in the β (beta)of individual security are not perfectly positively correlated, the β of portfolios can be much more precise estimates of the true β (Blum, 1968).

The study follow Fama and McBeth two pass regressionto test these asset pricing models.The Durbin Watson is used to check serial correlation and measures the linear association between adjacent residuals from a regression model. If there is no serial correlation, the DW statistic will be around 2. The DW statistic will fall if there is positive serial correlation (in worst case, it will be near zero). If there is a negative correlation, thestatistic will lie somewhere between 2 and 4. Usually the limit for non-serial correlation is considered to be DW is from 1.8 to 2.2. A very strong positive serial correlation is considered at DW lower than 1.5 (Richardson and smith, 1993).

According to Richardson and smith(1993) to make the model more effective and efficient the selection criteria for the shares in the period are: Shares with no missing values in the period, Shares with adjusted $R^2 < 0$ or F significant (p-value) >0.05of the first pass regression of the excess returns on the market risk premium are excluded. And Shares are grouped by alphabetic order into group of 30 individual securities (Roll and Ross, 1980).

3.4.2.1 Model for CAPM

In first pass the linear regression is used to estimate beta which is the systematic risk.

$$R_i - R_f = (R_m - R_f)\beta \qquad (3.1)$$

Where $R_i$isMonthly return of thesecurity, R_f isMonthly risk free rate, R_m isMonthly return of market and βis systematic risk (market risk).

The excess returns R_i - R_f of each security is estimated from a time series share prices of KSE-100 index listed shares for each period under consideration. And for the same periodthe market Premium R_m - $R_f$also estimated. After that regress the excess returns R_i - R_f on the market premium R_m - $R_f$to find the beta coefficient (systematic risk).

Then a cross sectional regression or second pass regression is used on average excess returns of the shares and estimated betas.

$$\hat{R}_i = \gamma_0 + \gamma_1\beta_1 + \epsilon \qquad (3.2)$$

Where λ_0= intercept, $\hat{R}_I$is average excess returns of security i,$\beta_I$isestimated be coefficient of security I and Є is error term.

3.4.2.2 Model for APT

In first pass the betas coefficients are computed by using regression.

$$R_i - R_f = \beta_i f_1 + \beta_{i2} f_2 + \beta_{i3} f_3 + \beta_{i4} f_4 + \epsilon \qquad (3.3)$$

Where Ri is the monthly return of stock i,R_f is risk free rate, β_i is the sensitivity of stock i with factors and ϵ is the error term.
Then a cross sectional regression or second pass regression is used on average excess returns of the shares on the factor scores.

$$\hat{R} = \gamma_0 + \gamma_1 \beta_1 + \gamma_2 \beta_2 + \gamma_3 \beta_3 + \gamma_4 \beta_4 + \epsilon_i \qquad (3.4)$$

Where$\hat{R}$ is average monthly excess return of stock I, λ = risk premium, β_1 to β_4 are the factors scores and ε_i is the error term.

3.4.3 Comparison of the Models

The next step of the study is to compare these competing models to evaluate that which one of these models is more supported by data.This study follows the methods used by Chen (1983), the Davidson and Mackinnon equation (1981) and the posterior odds ratio (Zellner, 1979) for comparison of these Models.

3.4.3.1 Davidson and MacKinnon Equation

CAPM is considered the particular or strictly case of APT. These two models are non-nested because by imposing a set of linear restrictions on the parameters the APT cannot be reduced to CAPM. In other words the models do not have any common variable. Davidson and MacKinnon (1981) suggested the method to compare non-nested models. The study used the Davidson and MacKinnon equation (1981) to compare CAPM and APT.
This equation is as follows;

$$R_i = \alpha R_{APT} + (1 - \alpha) R_{CAPM} + e_i \qquad (3.5)$$

Where$R_{i=}$ the average monthly excess returns of the stock i, $R_{APT=}$ expected excess returns estimated by APT, $R_{CAPM=}$ expected excess returns estimated by CAPM and α measure the effectiveness of the models. The APT is the accurate model to forecast the returns of the stocks as compare to CAPMif α is close to 1.

3.4.3.2 Posterior Odds Ratio

A standard assumption in theoretical and empirical research in finance is that relevant variables (e.g stock returns) have multivariate normal distributions (Richardson and smith, 1993). Given the assumptionthat the residuals of the cross-sectional regression of the CAPM and the APT satisfy the IID (Independently and identically distribution) multivariate normal assumption (Campbell, Lo and MacKinlay, 1997), it is possible to calculate the posterior odds ratio between the two models.In general the posterior odds ratio is a more formal technique as compare to DM equation and has sounder theoretical grounds (Aggelidis and Maditinos, 2006).
The second comparison is done using posterior odd radio. The formula for posterior odds is given by Zellner (1979) in favor of model 0 over model 1.
The formula has the following form;

$$R = [ESS_0/ESS_1]^{N/2} N^{K_0 - K_1/2} \qquad (3.6)$$

WhereESS_0iserror sum of squares of APT, ESS_1iserror sum of squares of CAPM, Nisnumber of observations, $K_0$is number of independent variables of the APT and K_1 isnumber of independent variables of the CAPM.As according to the ratio when;
R> 1 means CAPM is more strongly supported by data under consideration than APT.
R < 1 means APT is more strongly supported by data under consideration than CAPM.

IV. RESULTS AND DISCUSSION

4.1 Results of Descriptive Statics of Study Variables

Table 4.1: Descriptive Statics

Variable	Minimum	Maximum	Mean	Std. Deviation	Jarque-Bera test	Sig
KSE-100 Index	-0.11	0.14	0.020	0.047	5.558	0.062
Inflation	-0.01	0.02	0.007	0.008	1.345	0.510
Exchange rate	-0.07	0.04	0.003	0.013	1.517	0.467
Oil Prices	-0.24	0.11	0.041	0.060	2.474	0.290
Interest rate	-0.13	0.05	0.047	0.029	1.745	0.418

Table 4.1 displayed mean, standard deviation, maximum minimum and jarque-bera test and its p value of the macroeconomic variables of the study. The descriptive statistics indicated that the mean values of variables (index, INF, EX, OilP and INT) were 0.020, 0.007, 0.003, 0.041 and 0.047 respectively. The maximum values of the variables between the study periods were 0.14, 0.02, 0.04, 0.41, 0.11 and 0.05 for the KSE- 100 Index, inflation, exchange rate, oil prices and interest rate.
The standard deviations for each variable indicated that data were widely spread around their respective means.
Column 6 in table 4.1 shows jarque bera test which is used to checkthe normality of data. The hypotheses of the normal distribution are given;
H_0 : The data is normally distributed.
H_1 :The data is not normally distributed.
Table 4.1 shows that at 5 % level of confidence, the null hypothesis of normality cannot be rejected. KSE-100 index and macroeconomic variables inflation, exchange rate, oil prices and interest rate are normally distributed.

The descriptive statistics from Table 4.1 showed that the values were normally distributed about their mean and variance. This indicated that aggregate stock prices on the KSE and the macroeconomic factors, inflation rate, oil prices, exchange rate, and interest rate are all not too much sensitive to periodic changes and speculation. To interpret, this study found that an individual investor could not earn higher rate of profit from the KSE. Additionally, individual investors and corporations could not earn higher profits and interest rates from the economy and foreign companies could not earn considerably higher returns in terms of exchange rate. The investor could only earn a normal profit from KSE.