Detection The Causing Pathogens Of Bacterial Diseases Of Groundnut Crop, Growing In Rajasthan

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RAMESH CHAND MEENA

INDEX

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1

Introduction

Groundnut is inhabitant crop of new world. It arose from a hybrid among two wild groundnut species, *Arachis ipaensis* and *Arachis duranensis* (Krapovickas & W. C. Gregory, 1994). Groundnut referred to as peanut, monkey-nut or earth-nut. It is suppose that the dissemination of groundnut crop from South America to Africa, Asia and Europe probably between sixteenth to seventeenth centuries (Gibbons *et al.,* 1972). In India the groundnut crop was introduced from China (Kochert G. *et al.,* 1996).

Overall the world 109 countries cultivated the groundnut crop, on approximately 29.6 million hectares of which 4.8 million hectare in India. Total production on global label is 48.8 million tons, of which occurred about 95 percent in developing countries. On the global label an average yield production of groundnut was found 1.67 Mt ha^{-1} and in India was 1.46 Mt ha^{-1} in year of 2021. In the term of area of groundnut production, India occupies first position and in terms of production China (177.73 lakh tones) occupies first position

and India (67.35 lakh tones) occupies second position in the world in 2021 (FAOSTAT., 2021).

The demand of groundnut global production will needs to double in next year of 2050 according to rising world population which is rapidly increases year to year. Though, the estimates are far below by needed in present time. (Ray *et al*., 2012). Decrease the production of crops by weeds, diseases and attack of insects in world-level by approximately thirty six percent which alone plant diseases have been shown to loss of crop yields by approximately 14 percent (Agrison. G.N., 2005). So it is necessary for increased the crop production to control the plant diseases. Yield losses of production in term of annually by 10-20 percent from seed and soil-borne diseases (USDA., 2003).

However, according Agrison (2005) beneficial crops breeding, improvement the methods of cultivation, crop land expansion, increases irrigation methods and improvement the productivity of crops are all of possible methods of raising the groundnut production but it is necessary to control the diseases. To prevent by the harmful effect of chemical pesticides, it is necessary at the recent time bio-control agents should be used in large quantity.

The lower sowing area was reported in comparison of previous year during kharif season 2021 due to low rainfall in India (IOPEPC 2021). Major groundnut growing states which are Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu Maharashtra and Rajasthan are contributed approximately 80 percent production in India. After these states next to groundnut producing states are Madhya Pradesh, Uttar Pradesh, Orissa and Punjab (Groundnut Outlook., 2021).

In Rajasthan, ten districts which are Bikaner, Jodhpur, Churu, Jaipur, Hanumangarh, Sikar, Dausa, Alwar, Nagaur and Tonk found as major groundnut producing districts. The highest yield production (2463 kg/ha) was expected for Bikaner in Rajasthan. Contribution role in yield production of groundnut, alone Bikaner district contributed approximately 40 percent of production overall Rajasthan. Total groundnut shell production in 2021 for Rajasthan was recorded is 14,24,968 mt by the normal producing range of 2,388 kg per hectare (IOPEPC 2021).

Groundnut plant is a member of the Papilionaceae family which is also known as pea family. The nature of groundnut plants are a yearly herbaceous and it can grow between 30-50 cm tall. It has a small axis and the major part of the plant consists with the primary branches. Prostrate shape of groundnut plant in given by secondary and tertiary branches. (Putnam D.H., 1991). Optimum range of rainfall should be between 500-1200 mm for better growth of groundnut. (Kocchar S.L., 2009).

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The seeds of groundnut are a rich source of the vegetable protein. An important amino acid, cystine and some unsaturated fats found in it. Approximately 44-56 percent oil has found in the groundnut seeds (Worthington and Hammons, 1971). According to geographical area the groundnut, oil content has differs in quantity (Brown *et al*., 1975; Holaday and Pearson, 1974; Young *et al.,* 1974). The oil of groundnut is rich with omega 3 fatty acid and use for cooking items. The groundnut oil which uses in many ways as food and cooking, it called as oil King of seed crops (Reddy M.V., 1976). Approximately 22-30 percent protein content, important minerals and vitamins are found in groundnut seeds (Savage and Keenan, 1994). The quantity of carbohydrate is reported to 9.5-19 percent in the groundnut seeds (Woodroof, J.G. 1983).

In the previous time it has been reported that the pathogen *Ralstonia solanacearum* known within different part of the world by *Pseudomonas solanacearum*, *Bacterium solanacearum, Burkholderia solanacearum* (Bradbury, 1986; Richardason, 1990; Balogun and Fawehinmi, 2008; Rakib *et al.,* 2011) alongwith India (Sitaramaiah and Sinha, 1983; Chakravarty and Kalita, 2011).

According to Yabuuchi *et al* in 1995 the bacterial wilt diseases affect the production of groundnut all over world. The causal agent of groundnut bacterial wilt diseases is found the world-wide. It has approximately fifty plant family host ranges which are making difficult to control it (Kelman *et al*., 1994). It has been reported that in the previous time different countries such as

Philippines (Natural *et al.*, 1988) to be free of this diseases (Hayward 1986; Tomlinson and Gunther 1986; Vellupillai 1986).

Some factors like as climate, density of host plant, presence of antagonist in soil and soil types are responsible for variation in its harshness. Bacterial wilt of groundnut is extensively distributed in Indonesia. Favorable conditions for pathogen are causes the presence of severe wilt incidence and economically loss.

According to international reviewer the bacterial wilt diseases start to spread in Indonesia in the year of 1892 and observed the effect on groundnut in 1905 (Kelman A., 1953). It broadly distributed all over Indonesia (Machmud., 1986).

It exposed that *R. solanacearum* observed as a seed-borne pathogen in groundnut (Machmud and Middleton, 1991; Machmud, 1993; Dongfang *et al.,* 1994). It was isolated from the pod shells, funiculus, seed cover and embryo (Machmud and Middleton, 1991; Machmud, 1993). Growth of *R. solanacearum* is directly depends on presence of water content in groundnut seeds. It cannot able to survive less than 10% quantity of water in the seeds (Zhang et al., 1993). It was not isolated from one year old seeds (Zeng *et al.,*1994).

Approximately 38-100 percent occurrence of infection *R. solanacearum* was found in seeds of groundnut which were collected from different part of India. Optimum range of temperature for the growth of pathogen on the groundnut at 18- 22°C and

optimum humidity range at 30-40 percent were recorded (Chatterjee *et al.,* 1994).

In India, the disease It has been reported that According to Sagar *et al* (2010) the bacterial wilt disease of groundnut was found in India from some states such as Gujarat, Rajasthan, Kerala, Karnataka, Himachal Pradesh. Casual agent of bacterial wilt is devided into five races (based on ranges of host) (Buddenhagen *et al.,* 1962) and six biovars (on the base of utilization of disaccharides and hexose alcohols) (Hayward A.C., 1964).

During July to October of year 2019 different sample of infected plants and plant parts are collected to isolation and identification of cause's bacterial pathogens. For the collection the sample well-recognized diseased plants should be selected. The infection on the plant should be at early, primary and secondary stages with colonizing pathogen present in decaying plant tissue. Seed samples were divided in three categories which are asymptomatic seeds, moderately discolored seeds and shriveled discolored seeds for the experimental work in the laboratory.

According to Cook *et a1* (1989), the origins of biovar 2 of *R. solanacearum* is reported from Americas. He *et al* (1983) reported the biovar1, 3 and 4 from Asia. Biovar 3 and 4 are having more virulent properties than biovar 1. The results of detection and identification of isolates are shows biovar 3, of which have collected the infected plant parts from different groundnut growing region of Rajasthan.

Seedling symptom tests of groundnut seeds with the culture of *Ralstonia solanacearum* were recorded in petri-plate method. According to result of pathogenicity test it is verified that every isolates of *Ralstonia solanacearum* were have the capacity to cause symptoms of bacterial wilt disease on emerging stage of groundnut. All the isolates have better capacity to decay of growing tissue in veins of tobacco leaves. According to Dhital *et al* (2001) *R. solanacearum* have full capacity for the creation of hypersensitivity reaction in leaves of tobacco plant. According to above results it has been indicated that isolates of *R. solanacearum* have a proper secretion system which is doing hypersensitivity reaction, like other plant pathogenic bacteria on the leaves of tobacco.

Phyto-pathological effect on seedling was observed at cotyledonary leaves. In severe infection the Hypocotyls and transition zone showed rotting situation in severe infection but on apical part of plants symptoms were observed dropping leaves. Deeply infected seedlings were showed mortality symptoms as compared of asymptomatic seeds. Entire plant showed wilting symptoms in the infection of severe. The presence of bacterial strain showed resembling to *R. solanacearum* wilting symptoms.

Diseases transmissions were carried out by petri-plate, test tube and pot experiments. All According to all experimented seed samples which are collected from twelve sites of Rajasthan shown depply disease occurrence symptoms between ranges of 13 to 100 percent. The inoculums of seed borne pathogens in transmission of

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bacterial wilt disease have strongly affected the diseases development from seed to the emergent groundnut plant.

In Rajasthan some major affecting factors are supports to attack of bacterial wilt disease on groundnut which showed heavy loss of the crop at all the stages of growth right from sowing to harvest and storage. The disease causes heavy damage in sandy loam. It is rising as a major and wide spread disease in Rajasthan. The pathogen seems to have adaptability to higher temperature and the disease occurs during July-Sept, which is particularly harsh at pre and post emergence stages causing considerably losses to the yield.

Under favorable situations for the pathogen, the infected plants are shows wilting symptoms. The pathogens are returned to soil after spread the diseases and try to spread it to next plants. Pathogens are show fast growth and can kill healthy groundnut plant within the same growth period. In one growth period the pathogen can moves 01 meter by soil without the help of water movement. After two to three weeks of the planting wilt symptoms can be seen in the groundnut. First symptoms can be observed as drooping of some leaves in groundnut. Later than stages the plant may bend, dry, turned brown and finally die. The roots and pods of infected plant are become rotten. According to Mehan *et al* (1994) the bacterial wilt diseases are identify by discoloration in xylem and shows the bacterial ooze.

ISBN : 978-93-93166-91-3

Nature of the pathogen is recognizes as seed and soil-borne form. It can survive in the soil at the resting period. The definition of soil-borne pathogen is which can be able to spread the diseases via soil to host plant through inoculums. Thus the seed-borne pathogens are inoculums with the germination of seeds. Both soil and seed-borne pathogens are reducing the yield of production in groundnut (Viswanathan, 2000).

ISBN : 978-93-93166-91-3

2

OBJECTIVES OF THE STUDY

1. Extensive literature review carried out.
2. Field surveys will be carried out to study the symptoms and incidence of the diseases.
3. Collection of affected plants and plant parts.
4. Isolation of bacterial pathogens.
5. Characterization of isolated bacterial culture.
6. Effects of bacterial pathogens on the crop shall be studied.
7. Disease transmission phyto-pathological effects and toxicity shall be evaluated.

ISBN : 978-93-93166-91-3

3

WORK PLAN

First year

1. Extensive literature review carried out.
2. Field surveys will be carried out to study the symptoms and incidence of the diseases.
3. Collection of affected plants and plant parts.

Second year

Aspects 1-3 will be continued.

4. Isolation of bacterial pathogens.
5. Characterization of isolated bacterial culture.
6. Effects of bacterial pathogens on the crop shall be studied.

Third year

Aspects 4-6 will be continued.

7. Disease transmission phyto-pathological effects and toxicity shall be evaluated.

ISBN : 978-93-93166-91-3

4

REVIEW OF LITERATURE

Cultivation of groundnut in South America was being cultivated by 1000 B.C. as new world crop. After that dissemination of the groundnut crop in the sixteenth and seventeenth centuries from South America to Africa, Asia, Europe and Pacific Islands (Isleib *et al.*, 1994). The cultivation of groundnut is being between 40 degrees north and 40 degrees south of the equator, where found the daily temperatures are above than 20°C and average range of rainfall is 500 to 1200 mm.

In the structure of groundnut seed found primary root, a stem axis, two cotyledons, leaf primordia and hypocotyl. Hypocotyl is support to push the seed into the soil surface at the time of germination. So, it is notable that the emergence of groundnut is shows intermediate type among the hypogeal and epigeal. The length of taproot growth is reaching of approximately 10-12 cm within three to six days. Groundnut plant grows up to 12 to 65 cm high and found sparsely hair on stem. A total of three major stems are developed, of which main stem is derived by the terminal bud on

ISBN : 978-93-93166-91-3

the epicotyl and other two lateral stems are derived from the auxiliary buds of cotyledon. A healthy- taproot derived from groundnut seeds with many lateral roots. Lateral roots are arranged by four series of spirally branches on taproot with a large number of nodules. In the roots do not found any conventional root hairs, but in the axils of lateral roots the clumps of hairs are formed (Moss and Ramanatha Rao, 1995). Normally after three days of seed planting, lateral roots show on the seedlings (Gregory *et al.,* 1973). Growth of groundnut plant is slow in early stage but between 35 to 80 days after planting being observed more rapid growth (Ramanatha Rao., 1988).

Uncertain growth, prostate nature, annual life cycle, herbaceous and self pollinating habit have been found in groundnut plant. Approximately one to six percent percentage cross pollination was recorded due to typical flower structure (Duke, 1981; Coffelt, 1989). Later than thirty to forty days of planting the flowering has been started by the groundnut plant and after six to ten weeks, highest flower production was recorded.

Natural self pollination is being started around sunrise and complete up to within five to six hours. After fertilization, between five to seven days the tip of the ovary bearing from one to five ovules, creating the carpophores (peg or gynophores) bearing assistant floral parts (Ramanatha Rao, 1988). The gynophores penetrate more than 5 cm into the soil and quickly elongates with

ISBN : 978-93-93166-91-3

positively geotropic growth until the ovary become develop in form of pod (Ramanatha Rao, 1988).

During a long period, the flowering process being continues. Total numbers of pod which develop on the groundnut plant are affected by the rain fall. On the groundnut plant, pods are found in different stages of development at the time of harvest. According to Ramanatha Rao (1988) pegs are require a long time to being develop in mature stage after enter in the soil. The pod constricted the seeds about 1-5 in numbers by elongated sphere with the diverse reticulation on the surface (Gregory *et al*., 1973 & Ramanatha Rao, 1988). According to Ramanatha Rao and Murty (1994) pods completed the maximum size two to three weeks after enter in the soil, for develop the maximum quantity of oil within six to seven weeks and maximum quantity of protein five to eight weeks after.

The production of groundnut crop is affected from different fungal and bacterial diseases. Bacterial diseases are reported from different regions of world. Bacterial wilt diseases are identified by its specific symptoms on the plants. A flaccid appearance was observed on the leaves. Entire plants are shows complete wilt in the favorable situations of pathogen and in the unfavorable conditions, symptoms are increase by slow movement. Yellowish or white drops of bacterial ooze are shows in the vascular tissues of stem when it cut in vertical (OEPP/EPPO, 2004).

The bacterial cells are spread in all the tissues by the vascular bundle in the highly pathogenic plants. The lytic enzymes of pathogen which are occasionally use as extra cellular polysaccharides cause the rot of plant tissues (Timms-Wilson *et al.,* 2001). The extracellular polysaccharide causes a speedy wilt stage in the infected plant (Saile *et al.,* 1997).

The symptoms of bacterial disease shows in the stems change into necrotic with chlorosis and wilting leaves. Internal region of vascular tissue is visible in brown colour. Leaves are change into yellowish colour before the collapsing. At the finally stage, leaves turn into necrotic, desiccate and at last collapse (OEPP/EPPO, 2004). Sophisticate machinery has found in *R. solanacearum* for invasion the plant tissues with a specific capacity to develop at a low concentration of substrate (Van Elsa *et al.,* 2000).

According to Smith *et al* (1997) stems are shows brown or yellowish streak when the plants turn into stunted and chlorotic. According to Hay (2001) the occurance of bacterial ooze from a cut made on stem is a diagnostic key for identify the *R. solanacearum*.

In Ouelessebougou and Sikasso it reported in the year of 1999 (Thera, 2000). According to Thera (2006) it is reported by groundnut, potato, tomato, eggplant, tobacco and pepper in the present time (Thera, 2006).

Growth of *R. solanacearum* show fast in the clay soil and cause severe loss but found slow in sandy loam and red lateritic

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soils (Mehan *et a1.,* 1986). Abdullah et a1 (1983) showed in greenhouse studies that The diseases was grater severe at high clay content in the greenhouse conditions compared above the same humidity content. The combination of soil types and moisture levels are directly affected on harshness of bacterial wilt disease. According to Abdullah *et al* (1983) the severity of wilt disease was observed to increase extensively with increase in soil moisture.

In the growing season the presence of high temperature in the soil support increase the growth of bacterial wilt on the groundnut plants. The concentration of soil humidity and above than 20°C soil temperature with the depth of five cm are support to increase the disease growth. Continuous planting in the wet soils are support to speedy growth of disease. Those fields which deeply infested by pathogens are very important for screening the germplasm and create the resistance. The wilt disease of groundnut is higher severe in humid sandy soils, and lower in alluvial loam soils. Constantly fertilized the poor quality soils with the organic manure have a lower incidence of wilt diseases than those soils which fertilized with chemicals. For the growth of pathogen the favorable soil pH is occur between five to seven pH.

It is observed that alkaline can be suppressing the soil. In the north of Nanxiong and Scian county, widely distributed the soil between 8 to 9 pH with purple colour is constantly cropped with groundnut and tobacco, another host of bacterial wilt, and yet The incidence of wilt disease is contently observed less than 3% when

the cropped with groundnut and tobacco. Experiments with the cross inoculation have showed that the isolates of tobacco are able to produce the bacterial wilt disease in groundnut. Results of the bacterial wilt disease in potted plants of groundnut crops with pH 8 and 9 were recorded respectively 5 and 0% (FAO Soil Bulletin 38/1).

The bacterial wilt of groundnut is heavily spread in Indonesia compare than other crops (Machmud., 1986). The results of bacterial wilt disease in groundnut crops in China recorded widespread in sandy soil in the compare of other country but not show serious symptoms in clay soil (Ma and Gao, 1956; OCRI of CAAS 1977b).

Those groundnut plants which growing in sandy soil are shows higher percent of wilt incidence compare than other soil content. For example, in pure sand (100%) the bacterial wilt incidence was observed 95% in pure sandy soil but it occurred on heavy yellow loam only 5%. The bacterial wilt incidence also reached up to 21.8% In the experiment with 60% sandy soil + 40% heavy yellow loam the incidence observed about 22%, while in the experiment with 40% sandy soil + 60% heavy yellow loam it occurred 10% (Hou and Wang 1980). It also recognized by wet and warm regions disease. For the speedy growth of *R. solanacearum* the high temperature and moisture are favor conditions (Zhou and Liu 1962; Li *et al.*, 1981).

Growth of bacterial wilt exceeded rapidly up to 25°C soil temperature and high precipitation (Wang *et al.,* 1983). Although the

soil moisture is the needy factors for the wilt growth but it affected in low quantity compare to soil temperature. Soil temperature is reduced by the high moisture and heavy precipitation, which are being down the growth of bacterial wilt (Li *et al.*, 1981; Wang *et al.,* 1983).

At the beginning of bacterial wilt disease the general symptoms are shows such as day wilting with recover at night, bronzing of leaves and discoloration of vascular tissues at the above of stems. To the identity of *R. solanacearum* the test of stem cut shows white ooze of bacterial exudes coming out from vessels when observed in the water. The leaves are during wilt disease not shows any change of colour.

In the stems section after harvesting of crops wilt symptoms of white bacterial oozing observed in vascular necrosis (OEPP/EPPO, 2004). To detect *R. solanacearum* should be develop the accurate, simple and rapid methods by the crop and soil (French *et al.,* 1995).

Traditional techniques are applied for the identification of bacterial colonies with biochemical characteristics and pathogenicity tests on the isolation base of pathogen which are colleted from seeds and plant parts on semi selective media during the culture.

According to Elphinstone *et al* (1998) in the temperate countries the pathogen of bacterial wilt find in the latent form with

lack of any symptoms on the weeds such as *Solanum dulcamara* which are found along with waterways.

Kelman'stetrazolium media is generally uses in the symptomatic plant material isolation. Selective media is use in the presence of secondary infections. Serological agglutination test is use in the sideways flow device and the bacterial streaming test can be used in the disease characters (Danks and Barker, 2000).

For the identity of bacterial wilt, bacterial streaming test is normally use easy and helpful. In the process of bacterial streaming test the infected plant stem cut above the soil line and placing it in a beaker of water. If white bacterial ooze is shows streaming out from the vascular bundles of stem, is recognized for *R. solanacearum* (OEPP/EPPO, 2004).

On the culture media different physiological and morphological characters of pathogen can be experimented. Beside it the pathogenicity test is also testedon this culture. Typical symptoms of pathogen should be seen within four days in pure inoculums culture (OEPP/EPPO, 2004).

The isolation of *R. solanacearum* is very difficult for those unexperienced researchers which have a little experience (French *et al.,* l995). Mutation rate of *R. solanacearum* in the culture is occurred very high so use the practical way to store it in the water. Some fermentation tests are normally used for recognition of *R. solanacearum* biovar. Several bio molecular techniques such as

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serological test and polymerase chain reaction test are also available for a fast identification at the present time (Alvarez, 2004).

For the study of epidemiology diagnose the diseases above media are used. While promoting growth of certain groups of the desired microorganism it prevented the growth (Rudolph *et al.,* 1990).

To distinguish *Ralstonia solanacearum* during isolation among the other bacteria and determination of virulent groups from avirulents group is used TZCA media (Kelman, 1954). According to Rudolph *et* al (1990) the character feature of virulent colonies are shows is white with pink centre but avirulent colonies are shows dark red in colour. The TZCA media is prepared by combination of peptone – 10 gm/l, caseine hydroisolate – 01 gm, agar – 15 gm, glucose – 5 gm, triphenyltetrazolium – 10 ml.

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5

STUDY AREA AND CLIMATOLOGY

Brief Geography of study area

Rajasthan is named by land of kings which have a large geographical area. Rajasthan located in western part of India. Of which total geographical area of our country, Rajasthan covers about 10.4% or 342239 square km area. Rajasthan have first position on the base of area and seventh position on population base in India (Boland C and David. L., 2003). The state of Rajasthan have a Great Indian Desert which recognized by Thar Desert. The border of Rajasthan state shares in west with Pakistan country. The border of Rajasthan is around by total of five Indian states which are Punjab in the north side, U.P. and Haryana in the northeast side, M.P. in southeast side and touch with Gujrat in south side. The position of Rajasthan state on the basis of geographical situation is 23.3 to 30.12 on the latitude of north side and 69.30 to 78.17 on the longitudes of east side. Throughout southern part of Rajasthan the Cancer Tropic is being cross (ISCS 2018).

Bikaner, Jaipur, Jodhpur, Churu, Sikar, Nagaur, Chittorgarh, Hanumangarh, Dausa and Alwar districts are role a major part in the groundnut production in Rajasthan. On the base of yield production of groundnut the highest production observes with 2456 kg/ha was suggested to Bikaner district in the Rajasthan. According to report of IOPEPC (2019) the total production of in shell groundnut was reported at 14,05,781 metric ton with an average of 2380 kg/ha production in Rajasthan.

Location of the study area

Study of areas for the collection of sample have been chose nearby area of Bikaner, Jodhpur, Sikar, Jaipur, Alwar and Dausa districts of Rajasthan. From each district has been selected two study area (tehsil) for collection the sample. Nokha and Kolayat from Bikanr, Mandor and Mathania from Jodhpur, Shrimadhopur and Khandela from sikar, Bassi and Chaksu from Jaipur, Baswa and Bandikui from Dausa, Rajgarh and Thanagazi from Alwar are selected study areas.

An area of one square meter was determined from the field at five spots on the every selected tehsils. Disease incidence was calculated in the percentage of every selected field by the blow mentioned formula.

Percent wilt incidence (PWI) = [(number of diseased plants in each field/total number of plants) x 100]

All those plants which are showing symptoms of bacterial wilt diseases are count as wilted plants. Severity of the wilt disease

was discussed with farmers in the regarding of disease incidence during the field survey.

Agro climatology

Normally groundnut crop is cultivated in warm regions of the world. Tropical regions which have a long warm period in a season are available as better groundnut growing area according its requiring conditions. For the healthy growth of groundnut crops should be some favourable climate conditions are required such as between 27 to 30 ºC temperature and 450 to 500 mm rainfall during the groundnut growing season. Better tolerance capacity has been observed in the groundnut crops against both drought and flooding conditions after established.

Essential rainfall

For the commercial production of groundnut let be use between 450 to 950 mm rainfalls but in minimum rainfall between 350 to 450 mm is also sufficient for the normal conditions. In the Rajasthan due to irregular rainfall approximately 65% production has been affected. According to Reddy *et al.,* (2003) the long period of dry spell and low range of rainfall during the period of groundnut cultivation are suggested the major reasons for low production in India.

According to Sindagi and Reddi (1972) the production of groundnut is being vulnerable due to rainfall disparity from each year in India. Rainfall variability in India during July to September is affected approximately 85% groundnut production Bhargava et al

(1974). Challinor *et al* (2003) reported by the analyzing study throughout 25 years of groundnut production approximately 50% decrease in India by rainfall in every season. Gadgil (2000) reported by the study that the variation in rainfall during the groundnut growing season in production.

For the healthy growth of groundnut crop approximately 500 mm seasonal rainfall is required in India (AICRPAM., 2003). Whereas, Popov (1984) and Ong (1986) reported by the study of relationship between yield and productivity that the production of groundnut is directly affected by the quantum of rainfall in compare of its distribution.

Temperature

For the healthy growth of groundnut optimum temperature is required between 25 to 30ºC (Weiss 2000). According to Cox (1979) growth of groundnut crop is dominantly controlled by factor of temperature. Temperature has been categorized in following ranges (i) base temperature (8-11.5ºC) (ii) optimum temperature (29-36.5ºC) and (iii) high range temperatures (41-47ºC). These categories of temperature are explained respectively as base temperature - temperatures range which support to growth and development of groundnut crops, optimum temperature - temperatures range which support to highest growth of crops and high range temperatures - temperatures above which affected by growth decrease. Mohamed suggested a cardinal temperature by the study of groundnut seed germination in year of 1984 for fourteen groundnut genotypes. According to Angus *et al* (1981) during vegetative growth the base

temperature was suggested between 3 to 10ºC range to be maximum than reproductive phase.

According to Leong and Ong (1983) the growth of groundnut is being conservative by the base temperature. For the seed germination and leaf appearance the most favourable temperature is required between 23 to 30 º C. Williams et al. (1975) reported that the most favourable temperature For the vegetative growth of groundnut crops there are optimum temperature required between range of 25-30ºC and for the reproductive growth it is required between 20-25º C.

Soil

For healthy growth of groundnut crops the sandy loam soil support in more quantity in the compare of other soils. Sandy soil is found as light soil form which helps in easy penetration of pegs and their growth. Between 6 - 6.5 soil pH range is being increased the productivity of groundnut in the compare of more or less range of pH. Total of four types soils are mostly support to the growth of groundnut in Rajasthan which are named as desert, Dunes, brown and sierozems soil.

For the control of bacterial disease of groundnut ethno-botanical study areas have been selected throughout Alwar and Dausa districts. A total 07 villages (Ajabgarh, Tehla, Tilwar, Sainthal, Arniya, Gullana and Kolwa) selected for ethno-botanical studies. The survey was conducted with the help of local farmers. For the control of bacterial disease we will applies some research

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step which are categorized as isolation and identification of pathogens, apply the bio-control agent on identified pathogens and observe the effect of bio-control agents.

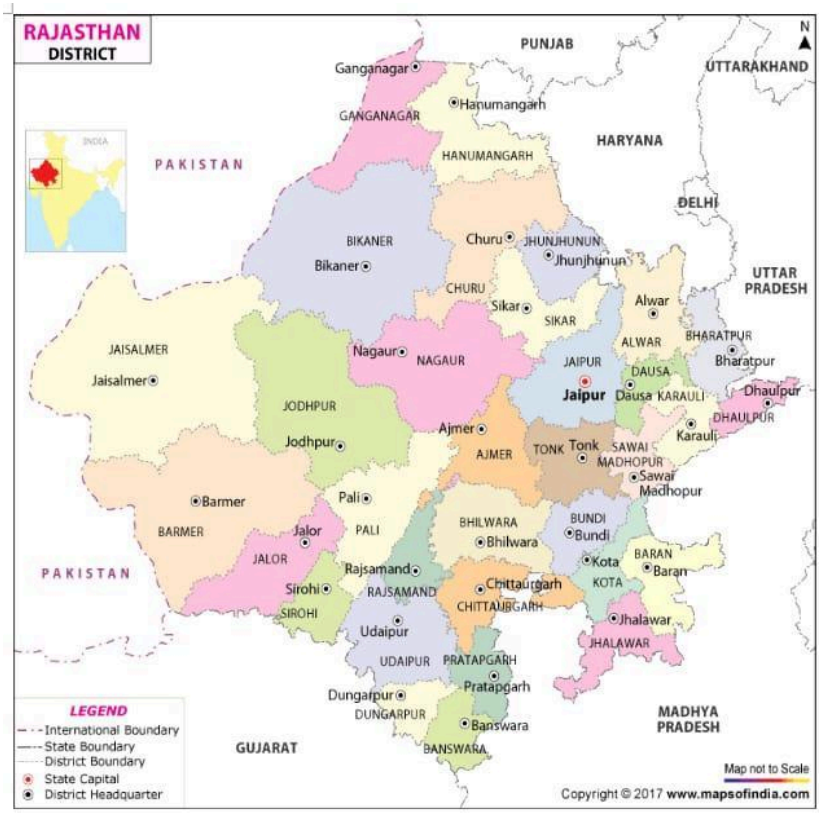

Figure- 1: Geographical Map of Rajasthan

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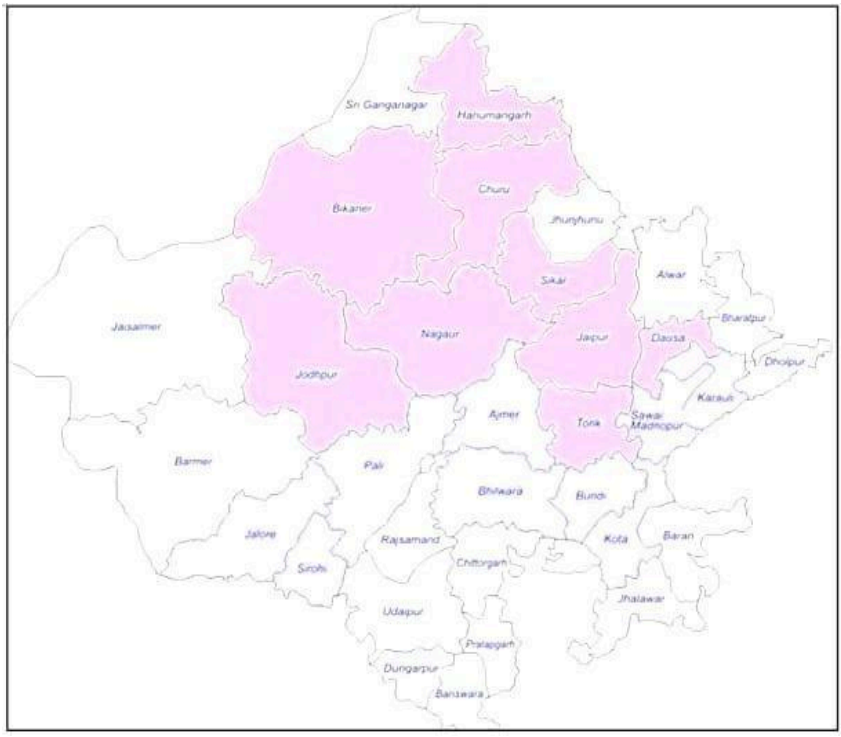

Figure- 2: Show Major Study Area Surveyed in Map of Rajasthan

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6

MATERIAL AND METHODS

Field surveys and collection the samples of seeds and plant parts

To know the status of bacterial diseases of groundnut, a survey was carried out the different districts of Rjasthan in the groundnut growing season. We have selected a total of 12 sites for collection the samples of wilted plant parts of groundnut. These sites selected through Bikaner, Jodhpur, Sikar, Jaipur, Dausa and Alwar districts. From each district have been selected two sites (tehsil).

Table-1: Samples collection Place (tehsils) in different districts and their code number.

S.N.	Tehsil	Code no.
1	Bassi	AT -01
2	Chaksu	AT -02
3	Mandor	AT -03
4	Mathania	AT -04
5	Shrimadhopur	AT -05
6	Khandela	AT -06
7	Nokha	AT -07
8	Kolayat	AT -08
9	Baswa	AT -09
10	Bandiki	AT -10
11	Rajgarh	AT -11
12	Thanagazi	AT -12

Nokha and Kolayat from Bikanr, Mandor and Mathania from Jodhpur, Shrimadhopur and Khandela from sikar, Bassi and Chaksu from Jaipur, Baswa and Bandikui from Dausa, Rajgarh and Thanagazi from Alwar are selected sites. Samples have been collected within various intervals during July to October of year 2019. All Diseased plants are selected on the basis of well-identified characteristic features. We should carefully select those plants which have infection at in early stage.

The invaders are being decayed the plant tissues during primary to secondary stage and colonized in plant tissue. Naturally infected groundnut seeds and other plant parts have been collected at the various growth stages plants from different farmer's fields. Among the each field total of five samples of diseased plants have collected. Essential information such as date, place, plant growth stage, variety is mention after its packing. No more time should be taken between sampling and laboratory processing. So, all the preparations of laboratory should be done before the process of sampling. Collected seed samples and infected plant parts from various farmers fields are come to laboratory for the isolation of the casual agents.

Evaluation of severity and wilt incidence

Evaluation of severity and wilt incidence were being done on the base bacterial wilt disease in groundnut. At least two locations are selected for the recorded the data of disease incidence from each groundnut growing region. Wilt incidence in form of percent was evaluated by the following formula which is given below:

Percent of wilt incidence of Bacterial wilt (PWI) = [(number of wilted plants in each field/total number of growing plants) x 100]

For the evaluation of wilt incidence in each groundnut growing area randomly five plants are choose from groundnut growing fields. According to Horita and Tsuchiya (2001) for the

calculation of wilt severity a measurement range is described as that no symptoms are present-1, apical leaves infected-2, two infected leaves present-3, more than four infected leaves are present-4 and entire plant shows dies than-5 severity scale.

Isolation and identification of bacterial pathogens

By the following procedure can be isolated the pathogens of bacterial disease from the diseased plants:-

Collected seed samples are categorized for the easy practical process respectively as symptomatic seeds, moderately discolored seeds and shriveled discolored seeds. The surface of infected seeds and other plant parts are sterilized. Infected plant materials have been cut into piece of 3 to 4 cm long. After than these plant materials are wash by sterile water and dry them by the blotting papers. All these plant materials are individually placed in 5 ml test tubes which are containing by sterile distilled water. Bacterial pathogens are observed in form of ooze from the cut ends within a little time. A loopful of the bacterial suspension has been taken after three to five minute and streaks it on the medium of nutrient agar. For the incubation these plates are placed at least 24 hours on temperature of 28 º C.

According to Kelman (1954) the groups of casual agents were purified on TZC medium by the streaking of single colony of every isolate. For the pathogenicity test a single colony of virulent isolate of of *R. solanacearum* has been selected which are showing

irregular in shape and whitish colour with pink central part. The pathogenicity test has been done by the inoculation method on the seedling of one month old tobacco plants to conform the isolates.

Every isolate which are representing a group of *Ralstonia solanacearum* bacterial suspension was injected in the leaves of tobacco at the one month old seedling. By a hypodermal syringe the bacterial suspensions has been injected into intracellular gap of the leaves. Continual up to five days observed the hypersensitive reaction for infiltration. On the -20 ° C temperature conditions the isolates of *Ralstonia solanacearum* were preserved for further biochemical experiments in 10% skim milk.

Characterization of bacterial pathogens with the biochemical tests:

Test on semi-selective agar media

According to Kelmen (1954) for observe the incidences of *R. solanacearum* the collected seed samples and other plant material of diseased plants are cultured on Nutrient Agar (NA) medium. Lather than approximately 72 hours the pure typical colonies of bacterial isolated which are growing on Nutrient Agar (NA) medium were transferred on tetra zolium chloride (TZC) agar medium plate to recognized the virulence of bacterial pathogen. According to Kelman (1954) the characteristic feature of a virulent colony shows whitish colour with pink centre part while the avirulence colonies

shows only whitish cream colour strains of *Ralstonia solanacearum* were recognized by tetra zolium chloride agar medium.

Recognition the nature of isolates

Differentiation between virulent and avirulent isolate of *Ralstonia solanacearum* have been done by the test of tetra zolium choloride agar medium. The virulent isolates are recognize on TZC medium after incubation at 24 hours by characteristic features of whit ish colour with pink centre part and avirulent isolates are recognige by off-white cream colour colonies.

Characterization by the test of gram staining

The bacterium suspension was taken by a dropper and spread it on a glass slide. After that it preset by minimum flame of heat and make a smear. Later than on the smear of bacterial suspension spread the aqueous solution of crystal violet for thirty seconds. And later than by the tap water it washed for one minute. After this it was stained by the iodine solution for one minute and then washed in tape water. Later then washed it by ninety five percent ethil alcohol still runoff it's less the colour. At the last bacterial suspension was about ten seconds stained by safranin and observed it by the help of microscope (Schaad,1980).

Experiments with LOPAT:

(1). Production of levan by the use of sucrose: Use the nutrient agar medium with five percent sucrose for the culture of bacterial

suspension of *R. solanacearum*. Later then these cultured plates are incubated for two days at the temperature of 28°C. Productions of levan by the growing bacterial colonies are noticed (Schaad, 1980).

(2). Experiment for Kovacs oxidase: Approximately 100 ml solution of oxidase reagent which is generally use one percent tetra-methyl-p-phenyl diamine dihydrochloride take in a dark bottle. A piece of filter paper with some drops of oxidase reagent is used on the petri dish. On the filter paper which have one percent solution of oxidase reagent take some bacterial suspension for the inoculation. The suspension of bacteria is observed after ten to sixty seconds in changing of purple colour.

(3). Experiment with potassium hydroxide: Bacterial suspension which are growing on cultured media take a drop on three percent potassium hydroxide solution and noticed after ten seconds for slime thread growth (Suslow et al., 1982).

(4). Experiment with Arginine dihydrolase: Take a 5 ml quantity of bacterial suspension in the test tube which have 15 ml capacity with screw –binding and warming it's at the temperature of 100°C. After this the medium of bacterial suspension was inoculated with arginine. On about 45°C temperature inoculated the bacterial suspension in the sealed test tube with 5 ml molten and one percent difco bacto cooled. For the observation of presence alkaline change the pH after seven days at the temperature of 28°C in the seal of agar.

(5). Experiment for hypersensitivity in tobacco plants: In the tobacco hypersensitivity test was performed by hypersensitivity Effects on the leaf of tobacco plants are observed by the reaction of hypersensitivity. Hypersensitivity reactions are observed by symptoms such as presence of necrosis and turgidity in the leaves.

Recognition of different races and biovars:

For the recognition of biovar strains some biochemical methods are applied on the samples which are collected from different groundnut growing districts such Bikaner, Jodhpur, Sikar, Jaipur, Dausa and Alwar in Rajasthan. The capacity of these bacterial suspensions is experimented on the utilization of different disaccharides and hexose alcohols (Hayward., 1954 and He *et al.,* 1983). Changing the colour into yellow causing by the oxidation of the carbon source is distinguished the positive effects.

Denny and Hayward are reported in year of 2001 that no-carbon-added control on the basal media is moreover used to change the colour. To make the suspension from 24-48h old cultures every group of isolates was prepared by adding various inoculums of pathogen in distilled water containing about 108 CFU/ml. The suspension of bacteria has been incubated in the plate at the temperature of 27- 33°C.

According to Schaad *et al* (2001) by change the colour of test tubes were experimented next to three days after inoculation. On the range of extensive host for the recognition of the races of *R.*

solanacearum were experimented by pathogenicity test (Schaad et al., 2001). Until the development of symptoms in plants were kept in net house after the incubation.

Experiment by the fermentation of sugar

According to Hayward (1964) the identification of bacterial pathogen was done on the basis of fermentation. Acid production by the fermentation of sugar indicated the presence of aerobic gram – negative bacteria. For the sterility the solutions have been heated for half hour at the temperature of 100°C.

Test for pectolytic activity

For the study of pectolytic activity take some healthy groundnut seed and sterilized their surface with well wash then peeled it aseptically. In humid conditions incubated three groundnut seeds in sterile petri dishes. At the room temperature two groundnut seeds were inoculated with loopful of bacteria by each isolate and incubated it up to 24-48 hrs for the recognition of bacterial symptoms. Then observe the situation of groundnut seeds for perform of pectolytic activity.

Fluorescent pigment test

At the temperature between 28- 30°C incubated with inoculated bacterium plate for two days. After that these plates were studied under the UV light for the development of fluorescent pigment.

Test for the pathogenicity

Tobacco leaves are used for perform of pathogenicity test. For the pathogenicity test Single colony of bacterial wilt pathogen are multiplied on the medium of nutrient agar which are showing white colour, irregular and fluidal. After that filtered two days old culture of bacterial pathogens on the tobacco leaves. For the next test, up to one day dipped the seedlings of groundnut into suspension of bacterial pathogen and incubated for 72 to 120 hours for observation of symptoms at the room temperature. For the pathogenicity test some other crops such as tomato, capsicum and okra are used as host for isolate of *Ralstonia solanacearum.* The ability of bacterial pathogens is observed the reaction of infection on other crops than groundnut. To decide the nature of casual agent of the isolates of *Ralstonia solanacearum* to studied of hypersensitivity response on the leaves of tobacco crops into internal tissue by the infiltration. Calculated the wilt incidence in the form of percent after use above procedure and observation to prove pathogenicity were recorded by the following formula-

Incidence of wilt percent= [(total number of wilted plants/total studied plants) x 100]

Transmission of wilt disease

According to Machmud and Middleton (1991) wilted seeds are developed approximately five to eight percent wilted groundnut plants. Wilting of groundnut crops were produced by normally

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infected seeds. No evidence is available healthy plant obtained from wilted plants. According to Roopali Singh (1994) between seed infection and mortality of plant found no combination.

On the semi-selective medium two naturally contaminated groundnut seed samples show approximately more than 85% disease symptoms of *Ralstonia solanacearum.* In the alternation cycles between light and darkness up to ten days, a total of hundred seed samples are shown in moist blotters and test tube on the one percent agar medium at the temperature between 25- 30°C. And hundred seeds are shown in pot experiment with each category of seeds and observed the seedling symptoms and mortality between germination and ingermination percent of associated with casual agents. At the various stages plant developing carried out the isolation of casual agent from the diseased plants.

ISBN : 978-93-93166-91-3

7

RESULTS

Severity and occurrence

A total of twelve major groundnut growing areas have surveyed where the occurrence of bacterial wilt disease normally observed. These areas are (Nokha, Kolayat, Mandor, Mathania, Shrimadhopur, Khandela, Bassi, Chaksu, Baswa, Bandikui, Rajgarh, Thanagazi) selected from six major groundnut producing districts in Rajasthan.

ISBN : 978-93-93166-91-3

Table- 2: Severity and occurrence of wilt disease of groundnut producing region.

Name of surveyed regions	Occurrence of wilt disease in percentage	Scale of wilt severity between 1 to 5
Bassi	22.64	4
Chaksu	07.56	3
Mandor	10.67	3
Mathania	14.36	3
Shrimadhopur	09.98	3
Khandela	21.12	3
Nokha	06.16	2
Kolayat	17.60	3
Baswa	20.76	2
Bandikui	12.34	3
Rajgarh	11.18	2
Thanagazi	08.46	2

Above these regions occurred an important variation in the occurence of bacterial wilt disease. The bacterial wilt disease occurrences were calculated by the following formula and results have mentioned in the table- 02.

Occurrence of wilt disease (%) = [(number of diseased plants in studied field/total number growing of plants) x 100]

According to result of survey showed highest occurrence of bacterial wilt disease (22.64%) was observed in Bassi region and wilt occurrence 21.56 and 20.76 percent found in Khandela and Baswa respectively. Whereas the lowest wilt occurence 6.16% has noted in Nokha. In Chaksu, Mandor, Mathania, Shrimadhopur, Kolayat, Bandikui, Rajgarh and Thanagazi the occurence of bacterial wilt disease in groundnut were noted 7.56, 10.67, 14.36, 9.98, 17.60, 12.34, 11.18 and 8.46% respectively (Table 2).

For the calculation of wilt severity randomly selected five farmer fields from each determined area. Horita and Tsuchiya suggested a scale for the calculation of severity of wilt disease in the year of 2001. A measurement range is described as that no symptoms are present-1, apical leaves infected-2, present the two infected leaves-3, presented the four or above infected leaves-4 and entire plant shows dies than-5 severity scale.

According to Horita and Tsuchiya suggested scale, the highest disease severity was observed -04 in Bassi region, whereas the lowest disease severity was occurred -02 in Nokha, Baswa, Rajagarh and Thanagazi groundnut producing regions. And in Chaksu, Mandor, Mathania, Shrimadhopur, Khandela, Kolayat, Bandikui the severity of bacterial wilt disease of groundnut was noted -03 (Table 2).

ISBN : 978-93-93166-91-3

According to Chatterjee *et al* (1997) the severity and occurrence results are showing variation on the basis of area. Some are major factors such as environment situations, large range of host plant and wide geographical presence affected the severity and incidence of bacterial wilt disease in groundnut.

Isolation and recognition of bacterial pathogens:

Collected total of 79 isolate of *R*alstonia *solanacearum* from each wilted groundnut selected area. All the samples of isolates were given a specific identification number from SM-001 to SM-079 respectivly. Thirty one (31) isolates were obtained from Bassi, 4 from Chaksu, 5 from Mandor, 5 from Mathania, 5 from Shrimadhopur, 2 from Khandela, 6 from Nokha, 5 from Kolayat, 3 from Baswa, 8 from Bandikui, 2 from Rajgarh and 3 from Thanagazi (Table 3).

Though take same quantity of samples from every selected area but some samples are not isolate due to disease symptoms. So variations in the number of isolate have been found.

To recognition of the bacterial pathogens applied the isolates from every collected sample. All the isolates have produced cream colour colonies after one day which is growing on nutrient Agar medium.

ISBN : 978-93-93166-91-3

Table-3: Presence of bacterial pathogen in the groundnut seeds of various area of Rajasthan.

Name of Area	Total no. of seed samples	Infection percent in seeds from each Samples	Occurence percent on TZCA medium
Bassi	31	03.25-34.25	13-100
Chaksu	04	12.25-25.50	30-80
Mandor	05	06.75-14.50	20-90
Mathania	05	05.25-13.75	40-100
Shrimadhopur	05	08.75-19.25	30-85
Khandela	02	08.25-10.75	40-75
Nokha	06	05.25-34.75	40-85
Kolayat	05	06.25-21.75	80-100
Baswa	03	07.25-18.00	45-80
Bandikui	08	08.25-29.75	20-90
Rajgarh	02	11.25-17.50	30-70
Thanagazi	03	09.25-21.75	50-100
Total	**79**	**03.25-34.75**	**13-100**

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The surfaces of infected seeds are show deeply brown in colour and have semi-transparent shining area. Normally spitted coat on the infected groundnut seeds have occurred. The growth of *Ralstonia solanacearum* provided symptomatic characters during the incubation. The characteristic features of *Ralstonia solanacearum* like rod shaped and motile nature. *Ralstonia solanacearum* is an aerobically gram negative bacteria and not produce the endospores. The cells of *Ralstonia solanacearum* are shows without capsule and have 0.5-0.7 X 1.5-2.0 µm in size and are non-encapsulated.

On the Nutrient Agar medium the results are shows that all the tested isolates are develop the cream colour colonies which verified that the growing pathogens were *Ralstonia solanacearum* (Table 4).

Table- 4: Collected isolates were growing on nutrient agar medium.

Name of areas which isolates were tested	Culture on the medium of nutrient agar
Bassi	Colonies are shows with off-white colour
Chaksu	Colonies are shows with off-white colour
Mandor	Colonies are shows with off-white colour
Mathania	Colonies are shows with off-white colour
Shrimadhopur	Colonies are shows with off-white colour
Khandela	Colonies are shows with off-white colour
Nokha	Colonies are shows with off-white colour
Kolayat	Colonies are shows with off-white colour
Baswa	Colonies are shows with off-white colour
Bandikui	Colonies are shows with off-white colour
Rajgarh	Colonies are shows with off-white colour
Thanagazi	Colonies are shows with off-white colour

All these isolates have preserve at the temperature of -20°C and divided into seven groups for further biochemical experiments.

Recognition of bacterial pathogens by the biochemical test:

Test with semi-selective agar media of bacterial pathogen:

On the King's medium B the bacterial isolates produced fluorescent pigment on the fluorescent experiments. And all those isolates which are not able to produce the fluorescent pigment on the medium of King's B were transferred to medium of tetrazolium

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chloride agar (TZCA). The medium of tetrazolium chloride agar is prepared by the following combination such as peptone -10 gm, content of casein hydrolysate -1gm, content of glucose -5gm, agar-agar powder -15gm and 2,3,5-triphenyl tetrazolium chloride -0.05gm in one liter of distilled water. Before pouring of tetrazolium chloride agar into the petriplates mixed one ml solution per hundred ml of sterilized molten media at the temperature of 60°C. Incubated the all these petriplates at the temperature of 30°C for three to four days which have inoculated by pathogens.

ISBN : 978-93-93166-91-3

Table- 5: Occurrence of *R. solanacearum* on the medium of tetrazolium chloride agar.

S. No.	Classified Group	Occurrence on the medium of TZCA
1.	Group 1	Shows irregular, fluidal and whitish pink centre colony
2.	Group 2	Shows irregular, fluidal and whitish pink centre colony
3.	Group 3	Shows irregular, fluidal and whitish pink centre colony
4.	Group 4	Shows irregular, fluidal and whitish pink centre colony
5.	Group 5	Shows irregular, fluidal and whitish pink centre colony
6.	Group 6	Shows irregular, fluidal and whitish pink centre colony
7.	Group 7	Shows irregular, fluidal and whitish pink centre colony

Presence of whitish pink centre colony with irregular shape indicated the presence of *Ralstonia solanacearum* on the tetrazolium chloride agar medium (Mehan et al 1995) (Table-5).

Differentiation between virulent and avirulent isolates:

Differentiation between above mentioned strain of *Ralstonia solanacearum* has been carried out by the experiment on tetrazolium choloride agar medium. After the 24 hours of incubation on the medium of tetrazolium chloride agar avirulent colonies develop with off-white, small in size and non-fluidal features while virulent colonies shows with off-white with pink centre feature.

Table- 6: Recognition of isolates which are obtained from infected samples.

S.No.	Classified group	Occurrence on the medium of TZCA
1	Group 1	Colonies shows off-white colour with pink centre
2	Group 2	Colonies shows off-white colour with pink centre
3	Group 3	Colonies shows off-white colour with pink centre
4	Group 4	Colonies shows off-white colour with pink centre
5	Group 5	Colonies shows off-white colour with pink centre
6	Group 6	Colonies shows off-white colour with pink centre
7	Group 7	Colonies shows off-white colour with pink centre

According to Rudolph *et al* and Klement *et al* (1990) on the medium of tetrazolium chloride agar two types colonies were produced by *Ralstonia solanacearum* of which virulent colonies shows whitish colour with pink centres part.

All the isolates of *Ralstonia solanacearum* develops the white coloured colonies with the pink centre region on the experimented tetrazolium chloride agar medium which are collected from various groundnut producing regions. According to observation of these features showed that all the experimented isolate found in virulent nature (Table 6).

According to Kelman (1954) avirulent colonies of *Ralstonia solanacearum* are able to produce the fluorescent pigment on the King B medium while virulent colonies are not able to produce the

pigment. Whitish colonies with pink centres are produce on the tetrazolium chloride agar medium after incubation of 48 to 120 hours at the temperature of 28ºC Champoiseau (2008).

Gram's staining test for *Ralstonia solanacearum*:

Experiments on *R. solanacearum* with reaction of gram's staining were carried out by crystal violate. All the isolates did not show violet colour. Curved rod shaped structure and did not stained with crystal violet are indicate gram negative nature of experimented all group of *Ralstonia solanacearum* (Table-7).

Experiments with LOPAT:

(1). Production of levan by the use of sucrose

According to Avigad (1968) Levan is uses as of dextran which found in form of extracellular bacterial polysaccharide. In the presence of *R. solanacearum* Levan is performed by sucrose on the nutrient agar medium. Domed shaped colonies are produced by the isolate of *R. solanacearum* on the nutrient agar medium.

The production of levan sucrase (enzyme) was able to convert the substrate into levan and glucose on the containing sucrose substrate. *R. solanacearum* also use the substrate of sucrose for maintenance and growth during the process of fermentation (Table-7).

(2). Experiment for Kovacs oxidase

The oxidation capacity of *Ralstonia solanacearum* isolates is done by test of oxidation. Within few seconds all the isolate of *R.*

solanacearum are have capecity to produced dark blue colour with reagent of oxidation which verified the experimented isolate of *R. solanacearum* have gram negative nature (Table-7).

(3). Experiment with potassium hydroxide

Solubility of pottasium hydroxide test for *R. solanacearum* also verified the gram negative nature. According to Suslow *et al* (1982) for the differentiation between above these strains of *Ralstonia solanacearum* the KOH solubility method is easier than other traditional methods.

The gram positive bacteria show by an elastic thread and gram negative bacteria show viscous thread. According to above these results that all experimented group of isolates find the gram negative nature (Table-7).

(4). Experiment with Arginine dihydrolase

All the groups of *Ralstonia solanacearum* have capecity to dihydrolyse of arginine with the indication of changing the colour from pink to red within four days. To study of Arginine dihydrolase activity a fresh culture of each isolates were stabbed into agar tube and incubated at the temperature of 28°C.

Find the result that experimented all the groups of *Ralstonia solanacearum* were have a capecity to change the colour from pink to red which confirmed that the all the experimented groups shows gram negative nature (Table-7).

ISBN : 978-93-93166-91-3

Table- 7: Experiments for the identity of *R. solanacerum* by biochemical process

Bacterial isolates	Gram'sstaining test Gram'sstaining	KMB Fluorescent test	Gila-tin liqu-ifica- tion test	Pathogenicity and color test (TTC)	Levan formati-on test	Kovac's oxidase test	KOH Solu Bility Test	Arginine dihydrolysis	Tobacco HR test	Sugar fermentation test (Dextrsucros, Lactos	Inference
Group 1	-	-	+	-	+	+	+	-	+	+	*R. solanacerum*
Group 2	-	-	+	-	+	+	+	-	+	+	*R. solanacerum*
Group 3	-	-	+	-	+	+	+	-	+	+	*R. solanacerum*
Group 4	-	-	+	-	+	+	+	-	+	+	*R. solanacerum*
Group 5	-	-	+	-	+	+	+	-	+	+	*R. solanacerum*
Group 6	-	-	+	-	+	+	+	-	+	+	*R. solanacerum*
Group 7	-	-	+	-	+	+	+	-	+	+	*R. solanacerum*

(5). Experiment for hypersensitivity in tobacco plants: Later than leaves infiltration all the isolates of *R. solanacerum* shows positive indication in the test of tobacco hypersensitivity. Affected leaf tissues are lost the turgidity by desiccation after the penetration of 36 hours.

Test for the fermentation of sugar substrate

By the oxidation of sugar substrate that experimented all the groups of *Ralstonia solanacearum* have a capecity to change the colour from reddish to yellow. All these experimented groups are producing acids and gas by the fermentation of basic sugars such as sucrose, dextrose, manitol and lactose. By the fermentation of sugar shows changing colour from from raddish to yellow and producing the gas bubbles confirmed that all the groups of *Ralstonia solanacearum* able to oxidation of manitol (Table- 7).

Test for pectolytic activity

Sterilized groundnut seeds are used for the study of pectolytic activity and peeled them aseptically. Incubated total three groundnut seeds on the sterile petri dishes in a humid chamber. For the identification of soft rot symptoms inoculated two groundnut seeds with loopful of each groups for one to three days at the room temperature. Observation of the condition of groundnut seeds performed the activity of pectolytic test.

Presences of virulent strain of *R. solanacearum* are verified by the characteristic features which showing irregularly, fluidal and whitish pink centre colony develop on tetrazolium chloride agar

medium. A polar tuft of flagella is found in *R. solanacearum.* Diffusible brown pigment was developed but cell are non-fluorescent and not found any pigments. Curved rod shaped structure, elastic thread observed in KOH solubility test and gram negative nature has been verified the recognition of *Ralstonia solanacearum.*

A total isolates of *R. solanacearum* within few seconds have capecity to produce with regent of oxidase which verified that the result of oxidase test showed positive response. Under UV lights a fluorescent pigment is produced in the test of influorescence which shows non-fluorescent nature of *R. solanacearum. R. solanacearum* shows weak positive results in the test of gelatin liquefaction weak positive. Isolates of *R. solanacearum* have easily grown at the temperature blow than 40°C which have verified by test of temperature sensitivity.

Test for recognition of biovar and race

Use of sugar and alcohols has help to recognition of different biovars. All isolates have capecity to oxidize the disaccharides and alcohols between three to five days. The results of converting the colour are verified to reaction of oxidation. A total experimented groups of *Ralstonia solanacearum* have a capacity to change the colour from blue to yellow which indicate to race- I and biovar- III (Table-8).

ISBN : 978-93-93166-91-3

Table- 8: Identification the race and biovar of *R. solanacearum* by carbohydrate tests.

Name of isolates	Use of Maltose	Use of Lactose	Use of Sucrose	Use of Manitol	Use of Sorbitol	Use of Dulsitol	Identify Race	Identify Biovar
1.	+	+	+	+	+	+	Race-I	BiovarIII
2.	+	+	+	+	+	+	Race-I	BiovarIII
3.	+	+	+	+	+	+	Race-I	BiovarIII
4.	+	+	+	+	+	+	Race-I	BiovarIII
5.	+	+	+	+	+	+	Race-I	BiovarIII
6.	+	+	+	+	+	+	Race-I	BiovarIII
7.	+	+	+	+	+	+	Race-I	BiovarIII

Utilization of different sugar and sugar alcohols are differentiate particular race and biovars. Disaccharides and hexose alcohols are oxidize by biovar III whereas only hexose alcohols oxidize by biovar- I and only disaccharides oxidize by biovar II (Hayward., 1964; He *et al* 1983; and Kumar *et al* 1993).

Test for pathogenicity

Symptoms of growing infected seedling of groundnut are noted which have grown on test tube and petriplate with the mixture of *Ralstonia solanacearum.* All these experimented groups have a capecity to causing the bacterial wilt disease on the seedling of groundnut. Applications of all these isolates on the leaves of tobacco plants were able to cause of wilt disease and finally result showed the death of leaves. Dhital *et al.,* reported in the year of 2001 that *R. solanacearum* was able to create the hypersensitivity reaction in the leaves of tobacco plant. So it is confirmed that isolate of *R. solanacearum* have hrp –III secretion system which have induced the hypersensitivity reaction.

Phytopathological study of the wilted plants

In seedlings of groundnut plant observed primary symptoms such as oozing of basal leaves, apical part of leaves are showing brown colour. The hypocotyl zone was looking in form of rotting in the situation of severe contamination and apical part change into dropping. As compared to moderate infection the severe infections have a large number mortality of contaminated seedlings. Wilting

symptoms can be observed on the whole plant in the severe contamination. Occurrence of morphologically wilting symptoms in the early stage indicated to causing agent of *R. solanacearum*.

Transmission study of wilt disease

Experiment with culture on petri plate

A total of 79 samples are applying on the culture of petriplate process. Growth have been started after two days of incubation and after 8th day the presence the percentage of seed germination were 94, 78 and 57% in the sample of SM-008 and in the sample of Sm-013 observed the percentage of seed germination were 98, 81 and 61% respectively in the categories of asymptomatic (general), moderate (medium) and deeply discoloured (deeply infected).

All seeds which are not able to germinate are showing brownish, rotting and oozing of contaminate pathogen. In the sample of SM-008 seedling mortality have been found 3, 3 and 4% and in sample of SM-013 seedling mortality have been observed 1, 4 and 6% respectively in the categories of asymptomatic (general), moderate (medium) and deeply discoloured (deeply infected).

Experiment with test tube seedling symptoms

All those 79 seed samples are applying on method of test tube seedling symptoms. The results are observed after 15th days of incubation and found the percentage of seed germination on water

agar test tube were 83, 78 and 62% in the sample of SM-008 and observed in the sample of SM-013 were 82, 76 and 59% respectively in the categories of asymptomatic (general), moderate (medium) and deeply discoloured (deeply infected).

Cotyledonary leaves are observed brownish colour in the early stage. After 15th days the percentage of seedling mortality have been observed were 15, 28 and 33% in the sample of SM-008 and found in sample of SM-013 were 13, 25 and 27% respectively in the categories of asymptomatic (general), moderate (medium) and deeply discoloured (deeply infected).

In deeply discoloured seeds has found highest percentage of mortality in the compare of other two types in the samples of SM-008 and SM-013.

Experiment with pot process

Pot experiments apply with ten days old seedling and regularly to thirty day in affected after growing seed. Later than 30 days, Results are showing the percentage of seed germination was observed 80, 66, 58% in the sample of SM-008 and in the sample of SM-013 the percentage of seed germinations are found 79, 68 and 60% in SM-013 respectively in the categories of asymptomatic (general), moderate (medium) and deeply discoloured (deeply infected).

In the sample of SM-008 the percentage of seedling mortality were observed 5, 13 and 21% and in the sample of SM-013

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seedling mortality found 6, 14 and 18% respectively. Infected plants features are noted up to stage of fruiting. Symptomatic plant parts were surface Sterilized the infected plant parts and cultured on medium of nutrient agar which later than develop *R. solanacearum* isolates. All these symptoms are observed in fields during the survey.

In the early stage infected seedling showing rotting and browning symptoms in the root parts of plants and mortality showed in the last. In the petriplate methods the mortality has been found were 85 and 78.25% in deeply infected seedlings whereas 37.8 and 43.25% mortality found in test tube methods. Approximately within three days after of growth the cotyledonary are leaves showed rotting with brown colour in the situation of stab inoculation. On the fruits of infected plants the brown-sunken lesions are developed by casual agents of *R. solanacearum.*

Between (13-100%) incidences of *R. solanacearum* has been observed in the different seed samples which are collected from twelve groundnut producing regions of Rajasthan. Disease transmission of *R. solanacearum* play a major role by seed borne inoculations seed to seedling of groundnut.

Figure- 03 (A to N): Recognition and isolation of *Ralstonia solanacearum* in the contaminated seeds of groundnut.

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Moderate discoloured seeds are showing in figure A (upper left) and shriveled discoloured seeds are showing in figure B (upper right). Streaking the bacterial colonies around the seeds of groundnut on the nutrient agar medium are showing in figure C and D. *R. solanacearum* colonies which are develop the brown pigment on the nutrient agar medium are showing in figure E. Whitish pink centre colonies on tetrazolium chloride agar medium are showing in the figure of F and G. Results of KOH solubility and gram's staining test are showing in figure H and I. Results of test for dihydrolyase of arginine is showing in figure J. Soft rot symptoms test for groundnut are showing in figure K and L. Motality and groundnut seedling symptoms are showing in figure M and N.

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Figure- 4: Showing germinate seed on petriplate at eight days in SM-008

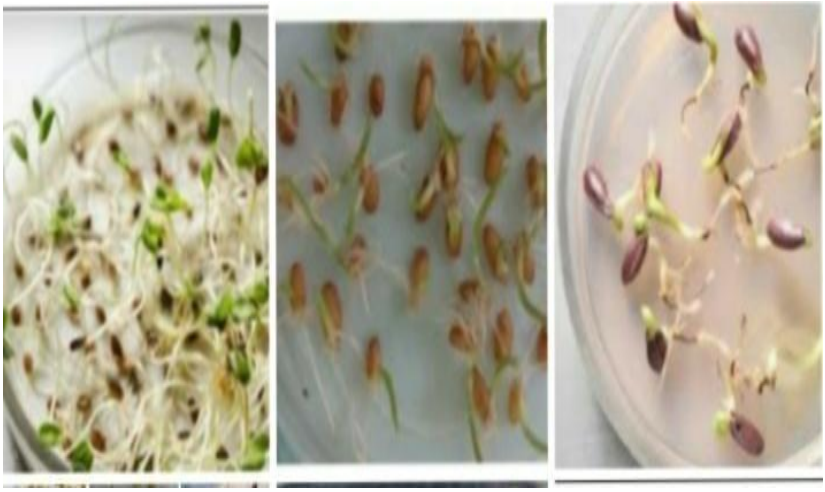

Figure- 5: Showing germinate seed on petriplate after eight days in in SM-013

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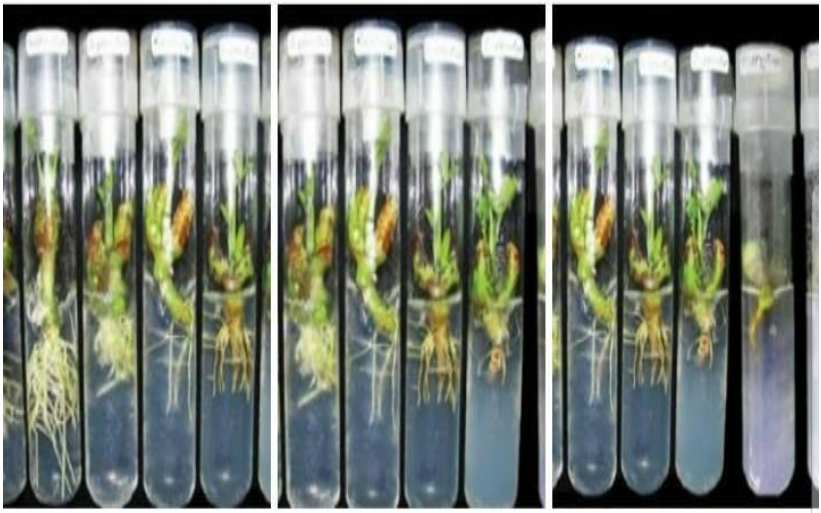

Figure- 6: Seed germination on water agar test tube after 15th day in SM-008

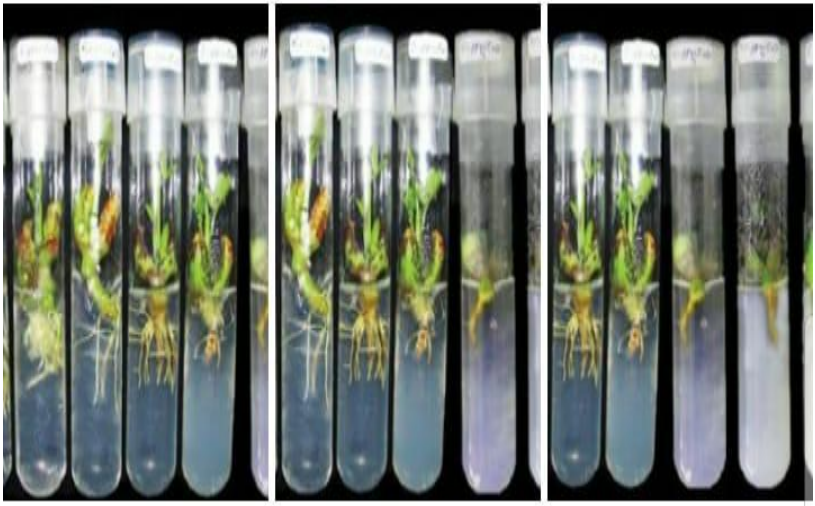

Figure- 7: Seed germination on water agar test tube after 15th day in SM-013

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Figure- 8: Seed germination on pot experiment after 30 day in SM-008

Figure- 9: Seed germination on pot experiment after 30 day in SM-013

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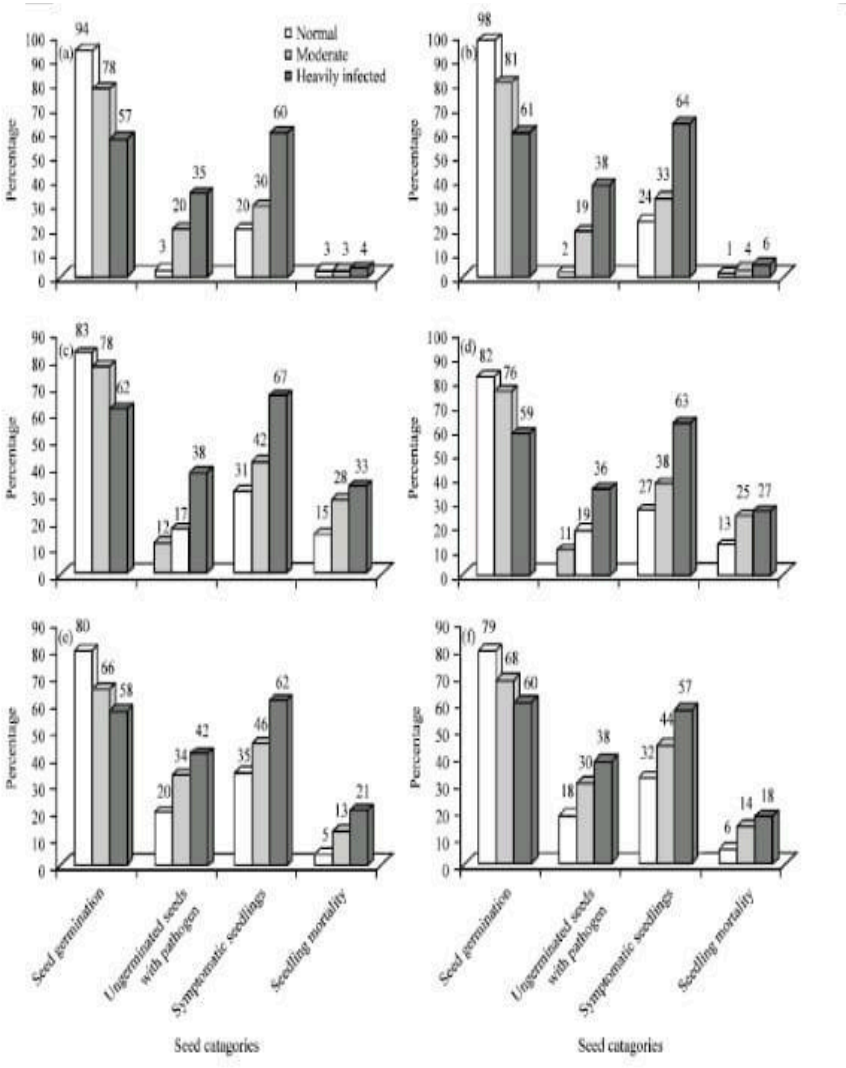

Figure- 10: Showing the result of *R. solanacearum* on the infected seedling of groundnut.

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Result of growing seedling in process of petriplate: (a) Tested seedling material- 08

(b) Tested seedling material-13

Result of growing seedling in process of test tube: (c) Tested seedling material- 08 (d) Tested seedling material-13

Result of growing seedling in process of pot test: (e) Tested seedling material- 08 (f) Tested seedling material-13

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8

DISCUSSION

In the hot humid tropical and temperate areas the bacterial wilt disease of groundnut is major harmful disease is observed in the world (Hayward AC., 1991). According to Yabuuchi E. *et al* (1995) the casual agent of *Ralstonia solanacearum* produced the bacterial wilt disease in groundnut crops. The casual agent *Ralstonia solanacearum* is observed by the all over world edding with India (Richardson MJ., 1990). From various groundnuts growing regions of Rajasthan collected a total of seventy nine diseased samples for the experimental studies. Above these areas selected through six major groundnut producing districts within Rajasthan. According to N.H.B.A. report (2005) for determination of the occurrence of wilting pathogen the experimental studies have complete by observation of dry seed, cultured by petriplate process of tetra zolium chloride agar medium and incubation on moistened blotters.

These petriplates are use for different experiments such as staining test, experiment of soluble with potassium hydroxide,

dihydrolysis test for arginine (Thornley MJ., 1960) test for development of levan, test for oxidation are tested on described plates (Hildebrand DC and Schroth MN., 1972). Used approximately one to two days old suspensions and culture of bacterial pathogen for done the entire test (Lelliot RA and Stead DE., 1987) (Kiraley Z., *et al.,* 1970).

Culture the total colonies of bacterial pathogen on nutrient agar medium which are isolated from various diseased groundnut seeds. Later then these colonies are transfered for the studies of levan formation to grow on five percent sucrose nutrient agar (SNA). According to Suslow TV *et al* (1982) the test with potassium hydroxide is better for identification between nature of bacteria than the recognition of strain of *Ralstonia solanacearum* by traditional method. In test of arginine dihydrolase casual agents of *R. solanacearum* observed negative reaction. According to research study of Sharma DK. (2007) found the same result on tomato, capsicum and tobacco. On the medium of King's B only levan negative colonies are developed.

The colonies of *R. solanacearum* was exposed as bacterium of non- fluorescent by the test of Gram's staining, King's medium B under light of ultraviolet and potassium hydroxide solubility. Thus the negative results of starch hydrolysis, kovac's oxidation and reduction of nitrate are confirmed that the experimented pathogen should be non-fluorescent bacterium. The tested isolates are easily developed on the medium of tetra zolium chloride which indicates that the

xanthomonads are not presented. The tested pseudomonas which confirmed as non-fluoresent bacterium is suggested to be *R. solanacearum.* Wilting symptoms on groundnut crops are also suggested to be occurrence of *Ralstonia solanacearum.*

According to Mehan VK and Mc Donald D (1995) are suggested the appearance of *R. solanacearum* on the medium of tetra zolium chloride agar by the showing of irregular, fluidal and whitish colonies with presence of pink center.

Lelliot R.A. and Stead D.E. (1987) are suggested by results of LOPAT test that experimented isolates recognized to be *R. solanacearum.* It was observed that seed Period In symptomatic seeds the process of germination have more time in the compare of asymptomatic seeds in the groundnut crops. On the hypocotyl regions various primary symptoms are noted like as brownish colour of cotyledonary leaves, showing the stalk reddish colour and found oozing. Hypocotyl is observed as rotting situation in the condition of severe infection. The percentage of mortality rate has been observed very high in the moderately infected seedlings of groundnut in the compare of asymptomatic seedlings. In the condition of severe infection the whole plant showed wilting symptoms such as rotting stalk, not flowering on plants and necrosis lesion on leaves (Ayers S.H. *et al.,* 1919).

Decrease the yield production of groundnut by the harmful effect of bacterial wilt disease is giving an account for a described study. According to Wang and Liang (2014) the control on bacterial

wilt disease is very difficult farmers due to improper management. The bacterial wilt disease has widely spread on groundnut producing regions. The using way for preventive of bacterial wilt disease are depends on the implementation and occurrence percentage.

Predominance of wilt incidence is observed from various groundnut producing regions are showed by studies in Rajasthan. In the groundnut growing areas in Rajasthan generate the difficult in the isolation and identification of isolates in the presence of maximum wilt incidence. The inoculation percentage is increase with the concentration of suspension of casual agent (Van der Wolf and De Boer, 2007; Baichoo and Jaufeerally, 2017). Accoerding to Rahman *et al* (2010) the isolation of *Ralstonia solanacearum* from the sample of groundnut seeds is easily done by culture on tetrazolium chloride agar medium.

Genotype and phenotype of *Ralstonia solanacearum* are observed in diversity by incidence and disease severity by the reason of its enormous geographical dispersal (Ahmed and Kerstin, 2011).

According to recent study the casual agent of *R. solanacearum* is prevails in all the surveyed regions of the Rajasthan state with different effective disease incidence. The prevailing agro climatic conditions and the nature of the host cultivar are some effective factors which are increased the severity and wilt incidence. The field survey is essential for the study of disease growth of bacterial wilt to know the development of *R. solanacearum* in groundnut crops. The identification of *R. solanacearum* was easily recognized by the technique of spread plate.

According to She *et al* (2017) the specific features of *Ralstonia solanacearum* on the culture of tetra zolium chloride agar medium recognized by the developing of whitish pink centered fluidal colonies. According to Jangir *et al* (2018) the avirulent colonies are recognized by the presence of deep red bluish border and virulent colonies are recognized by presence of pink enrered. The structure of *Ralstonia solanacearum* pathogen shows rod-shaped and gram- negative nature from various host plants (Afroz *et al*., 2011). Rod-shaped structure and gram-negative nature is also confirmed by the microscopic studies (Wang *et al.,* 2017; Ibrahim *et al*., 2019).

R. solanacearum have extremely fragile cell walls so it is creat slime thread. Different isolates of *R. solanacearum* give the various consumption results (Hayward, 1964). According to Rahman *et al* (2010) the formations of gasoline bubbles are verify the presence of both aerobic and facultative anaerobic bacteria. Through catalase test the isolates of *R. solanacearum* shows the formations of gasoline bubbles (Lual, 2017). According to Singh (2014) the isolates of *R. solanacearum* also shows positive reaction with oxidase test.

Deeply virulence capacity isolates of *R. solanacearum* is verified by the test of pathogenicity. All the collected isolates were identified as casual agent of *R. solanacearum* by the different test such as morphological, physiological and biochemical characterization.

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9

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