Integrated Management of Root-Rot of Soybean (*Glycine max* L.)

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Thesis

Master of Science in Agriculture

(Plant Pathology)

2011

DEPARTMENT OF PLANT PATHOLOGY

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This is to certify that **Mr. Jagdish Prasad Tetarwal** has successfully completed the Comprehensive Examination held on 30/05/2011 as required under the regulation for the degree of **Master's of Science** in Agriculture (Plant Pathology).

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This is to certify that the thesis entitled **"Integrated Management of Root-Rot of Soybean (*Glycine max* L.)"** submitted for the degree of **Master of Science in Agriculture** in the subject of Plant Pathology, embodies bonafide research work carried out by **Mr. Jagdish Prasad Tetarwal** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of this thesis was also approved by the advisory committee on …./…./2011.

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This is to certify that **Mr. Jagdish Prasad Tetarwal** student of **Master of Science in Agriculture, Department of Plant Pathology**, Rajasthan College of Agriculture, Udaipur has made all corrections/ modifications in the thesis entitled **"Integrated Management of Root-Rot of Soybean (*Glycine max* L.)"** which were suggested by the external examiner and the advisory committee in the oral examination held on / / 2011. The final copies of the thesis duly bound and corrected were submitted on / / 2011 are enclosed here with for approval.

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Enclose: One original and two copies of bound thesis forwarded to the Director Resident Instructions, Maharana Pratap University of Agriculture and Technology, Udaipur, through the Dean, Rajasthan College of Agriculture, Udaipur.

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Integrated Management of Root Rot of soybean (*Glycine max* L.)

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ABSTRACT

The present study was undertaken on integrated management of root-rot of soybean. The disease was recorded on popular cultivar 'JS– 335' with moderate to high infection around, Udaipur. The cultures of pathogens were isolated from the samples collected from the diseased plants received from Kolyari, Tehsil Jhadole , Udaipur. From these samples cultures of both *Rhizoctonia solani* (60%) and *Fusarium solani* (40%) were obtained and their pathogenicity was confirmed.

The seed transmission of *R. solani* and *F. solani,* was studied in five popular soybean cultivars viz., JS- 335, NRC- 76, JS 93-05, JS 97-52 and JS 95-60 using Blotter and Agar Plate method. Maximum recovery of both pathogens was in seeds of 'NRC- 76' and minimum in JS 95-60. The recovery of both the pathogens from both sterilized and un-sterilized seeds of all test cultivars, indicats external as well as internal seed borne nature of both the pathogens.

Seven fungicides at 500 and 1000 ppm concentrations, two BCAs and two neem formulations was first evaluated *in vitro*. Carbendazim and tebuconazole were highly effective at both the concentrations against *R. solani* and *F. solani in vitro* and were taken for further pot culture studies. Two neem based formulations (Neem oil 0.2% and *Azadirachtin* 0.2%), were found to be less effective against both the pathogen. Local isolates of *Trichoderma viride* and bacteria (*Bacillus* spp.), recovered from soybean rhizosphere showed high efficacy in suppressing both the pathogen in dual culture. The integration of effective fungicides and BCAs *in vitro,* were further evaluated as seed treatments individually as well as in different combination.

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Integration with carbendazim + *T. viride* + bacteria (*Bacillus* spp.) resulted in lowest disease severity (9.3% root-rot), maximum germination per cent (89.8%) and maximum dry weight (7.2g), as compared to their individual applications as well as over the untreated control. The disease suppression seemed to be due to reduction of the inoculum density of the pathogens.

These treatments are needed to be further tested in micro plots to ascertain their efficacy for practical disease control.

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1. INTRODUCTION

Soybean (*Glycine max)* is an important oil seed crop and is grown in several tropical and sub-tropical countries like USA, China, Brazil, Maxico and Russian federation. Soybean originated from China during 2800 B.C. It belongs to family leguminaceae and sub-family Papilionaceae. It contains about 43.2 % protein, 20.9 % carbohydrate, 19.5 % fat, 3.7 % fiber and small amount of vitamin B complex and vitamin E (Singh, 2010). It is used for preparation of soyamilk , antibiotics and cattle and poultry feed. Soybean oil being low in cholesterol has gained much attention from people suffering from heart problems.

The crop is commonly grown in *kharif* season. The main soybean producing states in India are M.P., U.P., Maharastra and Rajasthan. In India it ranks third in vegetable oil economy after groundnut and rapseed-mustard, and is cultivated on 9.33 million hactares with total production of 101.283 million tones and productivity of 1089 kg ha^{-1} (Anonymous, 2010-11). In Rajasthan it is mainly grown in Jhalawar, Chittorgarh, Kota, Bundi, Banswara, Udaipur and Bhilwara districts in 6.920 lakh ha. with production of 7.633 lakh million tones with average grain yield 1103 kg ha^{-1} (Anonymous, 2010-11).

Sustainable soybean production is continuously challenged by diseases that cause quantitative and qualitative losses in yield. About 20 pathogens including fungi, bacteria, viruses and nematodes, have been reported to attack soybean in India (Rangaswami and Mahadevan, 2008). Most wide spread among these are foliar diseases and root diseases. Foliar pathogens can be managed by the use of resistant varieties and / or judicious use of fungicides but root pathogens like *Rhizoctonia solani* and *Fusarium solani* being randomly distributed in soil and surviving as resistant structures, are difficult to control by conventional methods. Under favourable conditions these cause substantial losses and may become destructive at all the growth stages of the crop.

Root rot caused by *R. solani* and *F. solani* is known as one of the major yield reducer and economically important disease of soybean. In India root rot of soybean caused by *R. solani* by Singh *et. al*., (1974) and that caused by *F.solani* was first reported by Agarwal and Sarbhoy (1975). Limited information is available for management of

root rot caused by *R. solani* and *F. solani.* Preliminary surveys of soybean in Udaipur and Kota areas revealed that both *R. solani* and *F. solani* are involved together in causing this disease, and a control strategy to suppress both the pathogens is required. The popular cultivar JS-335 and NRC- 76 are susceptible to this disease. Application of fungicides is not very effective: when applied as seed treatment: these may not last longer enough in effective concentration to suppress the pathogen, while the crop remains susceptible to the pathogen, throughout the growing season. Soil application of fungicides is un-economical, hazardous and may also harm non-target (beneficial) flora in the soil. This problem can, however, be tackled through use of biocontrol agents or botanicals and their integration with fungicides. A good amount of literature is available on efficacy of fungal and bacterial biocontrol agents in suppressing *R. solani* and *F. solani* (Chet, 1989 and Cook and Baker, 1983) in other crops.

Efficacy and survivability of biocontrol agents is influenced by soil edaphic factors and associated microbiota. As such, use of local isolates of biocontrol agents is desirable. Studies were therefore, undertaken for developing an effective management strategy of root–rot complex in soybean with following objectives.

(1) To study the relative pathogenic potential of *Rhizoctonia solani* and *Fusarium solani.*

(2) To study efficacy of biocontrol agents, neem formulations and fungicides for suppression of *R.solani* and *F.solani in vitro*.

(3) To evaluate biocontrol agents, neem formulations and fungicides individually as well as in combination for suppression of Soybean root-rot in pot culture.

2. REVIEW OF LITERATURE

Root-rot of soybean caused by *Rhizoctonia solani* was first reported from Uttar Pradesh by Singh *et. al.,* (1974) where as, that caused by *Fusarium solani* was first reported from Delhi by Agarwal and Sarbhoy (1975).

The root rot of soybean is very wide spread and causes heavy losses in almost all the countries where soybean is grown. On the basis of three year`s studies with the several cultivars, Basu *et al*., (1976) reported that severely affected cultivars showed an about 57 per cent and moderately affected cultivars showed 35 per cent reduction in yield.

Pegg and Parry (1983) reported that soybean plants selected from fields at random were mostly free from aerial symptoms, but 90-100% showed browning of the lower stem and tap root were most commonly affected.

Michaud and Richard (1985) reported a significant difference in dry matter yield of 14 soybean cultivars grown for two years at three locations. All were affected by root rot (caused by *F. solani*) and most showing 20-30% infected seedlings. The frequency of disease free plants was less than 1.3%.

In a survey conducted in 71 soybean fields, Anderson *et al.,* (1988) found that *R. solani* and *F. solani* were isolated from 7 to 40 per cent stunted plants, respectively. Germination and emergence were 46% in plants infected by *R. solani* and 60% in areas with *F. solani* infection.

Hwang *et al.* (1989) surveyed soybean fields in southern Alberta and found that two species of *Fusarium*, *Fusarium solani* and *F. oxysporum* associated with soybean root rot, the incidence and severity were 61% and 80 % respectively.

On a survey of soybean fields by Kalb *et al.,* (1994) root rot was found in every field surveyed, with average tissue necrosis estimated at 21, 27 and 37% in plants from 1, 2 and 3 – year - old stands, respectively. *Fusarium oxysporum* and *F. solani* were the pathogenic fungi predominantly associated with necrotic roots, accounting for 25, 21 and 12 % of isolations, respectively.

Pratt and Wrather (1998) estimated the soybean yield loss of 9.29 %, 9.14 % and 8.67 % in the year 1994, 1995 and 1996 respectively. The greatest economic loss over the three year period was due to soybean root rot caused by *R. solani* and *F. solani* followed by *Phytophthora* root rot and charcoal rot.

No specific information about seed transmission of *Rhizoctonia solani* and *Fusarium solani* in soybean is available but these are generally known to be seed as well as soil-borne in crops like chilli (Holliday, 1981; Surekha *et al*., 1986).

Several factors influence the host, pathogen and environmental relationship which ultimately affect the intensity of the disease. Temperature is an important factor influencing growth, reproduction and survival of pathogen. Wolf and Wolf (1947) observed that most of the parasitic fungi grow within the range of 0^0C to 42 ^{0}C.

Relative humidity of 70 to 90 per cent showed good growth of the soybean root rot pathogen *F. solani*, whereas growth was retarded at 100 per cent relative humidity. 30 and 50 per cent relative humidity supported poor growth of the fungus (Kore and Kharwade, 1987). According to Ganacharya (1973) 80.5 and 21.5 per cent relative humidity was necessary for the maximum and minimum growth and sporulation of *F. solani*.

Saxena and Khare (1988) found maximum mortality of soybean crop induced by *F. solani,* at 25 % soil moisture which decreased with increase in moisture level. They reported severe incidence of wilt in sandy loam soil with 7.5 to 8.0 pH and temperature ranging from 20 ^{0}C to 30 ^{0}C.

Choi Kichun *et al.,* (1997) studied the effects of *F. oxysporum*, *F. solani* and *Rhizoctonia solani* on the growth of soybean seedlings. The pathogenicity of the fungi increased as the temperature increased. The emergence rate of seedlings decreased, when inoculated with the fungi. Chatuarvedi *et al.,* (2003) observed that the growth and sporulation of *Fusarium oxysporum* and *F. solani* were optimum at 25^0C.

A review of literature shows that the efforts to manage root-rot of soybean have been made largely through cultural practices. Whiting and Crookston (1993) found that soybean-maize rotation limited the build-up of host-specific pathogens of soybean, They

maintained the soybean in four cropping sequences ranging from annual alternation with maize to 1, 2 or 5 years of continuous cropping and found that soybean root rot caused by *R. solani* was prevalent during both years among less than 5% of all plants monitored.

Shalaby *et al.,* (1997) reported the intercropping of maize with soybean reduced the severity of root rot caused by *F. solani* and *R. solani* and stalk rot of maize than sole cropping conditions. It was concluded that microbial flora increased in the rhizosphere of maize and soybean under intercropping compared with sole cropping conditions.

Vallone (1998) reported that No-tillage cultivation of soybean resulted in increase in incidence of some diseases and reduction in that of others. Cultural practices which are considered to decrease the incidence of soybean diseases were crop rotation, use of resistant cultivar, sowing date, spacing and plant population, control of weeds and insects, use of fungicides and use of disease free seeds.

Seed dressing fungicides Benlate T (benomyl + thiram), Granson (benomyl + maneb), Vitavax SP (carboxin), Quinolate 15 M (copper oxyquinolate + imazalil) were found effective for control of soybean root-rot caused by *R.solani and F.solani* (Monem *et. al.,* 1984 and Arafa *et. al.,* 1997).

Various fungicides applied as seed treatment viz., Thiabendazole, Tolclofos Methyl, Benlate and Penycuron gave effective control of soybean root rot caused by *R. solani.* Of these Benlate (benomyl) was found superior for the control of this disease (Saharan and singh, 1994 and Gowily and Soliman, 1994). Sadowski (1994) reported that carbendazim and Benlate at 10 ppm in bioassay and as seed treatment @ 3 g / kg seed gave best control of *F. solani* from soybean.

Wahid *et al.,* (1995) reported that fungicides Derosal (carbendazim), Benlate (benomyl) at 10ppm and Vitavax (carboxin) at 50ppm gave complete inhibition and seed treatment @ 3g / kg was best for control of root-rot and collar-rot of soybean. Duan *et. al.,* (1996) also reported good effect of minerals and fungicides on the control of soybean root-rot.

Bavistin 50 WP (carbendazim) and Dithane Z-78 (zineb) completely inhibited the radial growth of *F. solani* and *R. solani* of soybean root rot were obtained

through soil drenching with carbendazim at 0.2 % Vadhera *et. al.,* (1997) and 0.15%, Prasad and Hiremath, (1985).

Gupta and Arora (1998) tested six different fungicides, copper oxychloride (blitox, 3 g/kg), captafol (foltaf, 3 g/kg), Dithane M-45 (mancozeb, 3 g/kg), Bavistin (carbendazim, 2 g/kg), captan (3 g/kg) and Thiram (3 g/kg) and found carbendazim was effective for the control of root rot of soybean caused by *R. solani* and *F. solani.*

Singh *et. al.,* (2000) tested the efficacy of 6 fungicides against *Fusarium solani in vitro*. The test fungicides were Baynate (thiophanate methyl), Blitox-50 (copper oxychloride), captan, carbendazim, Contaf (hexaconazole) and Indofil M-45 (mancozeb). All of the fungicides could inhibit fungal growth for upto 7 days of incubation. Blitox-50 and captan had fungistatic action while the other treatments were fungicidal. Vrataric *et. al.,* (2002) recommended fungicides Benit universal (imazalil + thiobendazole), Apron (metalaxyl), Vitavax (carboxin) for seed treatment and for foliar spray of (carbendazim + flutriafol) and Benlate (benomyl) for control of *Fusarium, Peronospora, Cercospora,* and *Phomopsis* causing soybean diseases.

Seed treatment of fungicides carbendazim, thiram and captan was found effective for control of soybean root-rot by *R. solani* (Wang *et. al.,* 2004 and Taya *et. al.,* 1990). Sun *et. al.,* (2006) determined the effect of silver carrying antimicrobial compounds (zeomic and AM1), for control of *R. solani, F.solani* and *F. oxysporum* in soybean.

Development of host plant resistance (HPR) provides a practical, environmentally sound and economic method to control soybean root rot. Shannon and Collins (2001) reported that soybean cultivar `DP-3588` developed by single selection exhibited resistance to soybean root rot pathogen *R. solani.*

Graham *et. al.,* (2002) used PCR amplification to identify a cluster of resistance gene analogoues (RGAs) on soybean linkage group 'J'. resistant to root rot of soybean. Burnham *et. al.,* (2003) identified simple sequence repeat (SSR) markers associated with putative quantitative trait loci (QTLs) for partial resistance to *R. solani* in soybean. Cooper *et. al.,* (2004) reported that F4-derived soybean line `Stalwart` was resistant to *R. solani.*

Salehi *et. al.,* (2005) used the bean chitinase gene which was cloned in recombinant binary plasmid vector pBI121 with 35s promoter of cauliflower mosaic virus, for the transformation of soybean using strain LBA4404 of *Agrobacterium*. Bioassey of chitinase activity of transgenic lines on *In vitro* condition prevented mycelial growth of *R. solani*. Sharma *et. al.,* (2005) reported that more than 50 soybean germplasm and breeding lines showed resistance to *R. solani*.

Three soybean cultivars PARS, BRAGG, and RAWAL were found tolerant to root infecting fungi *R. solani, F. solani* and *M. phaseolina* (Haque *et. al.,* 2007). Shannon *et. al.,* (2007) reported that S02-2259 soybean germplasm line from cross of Dp3519S x LG92-4208 showed resistance to soybean root rot disease.

There are several reports of effective suppression of these pathogen through biological control (Chet, 1989). The first report of biocontrol of plant pathogens by Weindling (1934) where it was reported that *Trichoderma lignorum* was antagonist to *Rhizoctonia solani* and other fungi. According to Marshal (1982) coating soybean seed with an aqueous suspension of *T. harzianum* resulted in 65 per cent reduction in damping-off caused by *R. solani*. Camporta (1985) reported that mycoparasitism by *Trichoderma* spp. was most the effective mechanism for destroying hyphae of *R. solani*. Mukhopadhyay and Kaur (1990) and Elias *et. al*., (1993) reported seed treatment with *T. harzianum* gave good control of *R. solani, F. solani, S. rolfsii* and *Pythium* spp. in soybean.

Biocontrol agents like *Trichoderma harzianum, Gliocladium virens, Paecilomyces lilacinus, Bacillus subtilis* and *Streptomyces* spp. have been reported to be effective for control of soybean root-rot pathogens (*Rhizoctonia solani, Macrophomina phaseolina, Fusarium. spp)* (Haque *et. al.,* 1990 and Mousa and Mousa 1994). Haque and Ghaffar (1993) reported that *Rhizobium meliloti, Rhizobium leguminosarum* and *Bradyrhizobium. japonicum* when used either as a seed dresser or as a soil drench reduced infection of *M. phasiolina, R. solani* and *Fusarium* spp. in soybean, mungbean, sunflower and okra crops.

Velikanov *et al*., (1994) observed a reverse correlation between hyperparasitic and antibiotic activities of *T. aureoviride* isolates 2288 and 1518 when tested against *F.*

oxysporum, *F. solani*, *P. ultimum* sp. *Rhizoctonia solani and Sclerotinia sclerotiorum* causing root rot of soybean.

Barbosa *et. al.,* (1995) evaluated the effect of soybean seed treatment with fluorescent *Pseudomonas* spp. for the control of *R. solani* and the potential of *Pseudomonas fluorescens* as a biocontrol agent was confirmed. A low correlation was found between *in vitro* and *in vivo* results.

Ghany (1996) reported good effect of *Azatobacter chroococcum* and *Azospirillum lipoferum* and its mixtures (1:1) for control of soybean root-rot by *R. solani* and *F. solani.* Mousa (1996) reported a marked resistance of soybean plants to root-rot caused by *R. solani* and *S. rolfsii* when the biocontrol agents *Bacillus subtilis* and *Trichoderma* spp. were introduced in the soil. Shawafdy and Mousa (1997) reported the *Parmelierea spp., Bacillus subtilis* and *Actinomyces griseus* greatly suppressed the *F. oxysporum* f.spp. *glycines, Sclerotium rolfsii, R. solani* and *Meloidogyne javanica* that cause root-rot or wilt of soybean.

Guo *et. al.,* (1998), reported two *Bacillus spp.* to be effective in controlling soybean root rot caused by *F. solani* and *R. solani*. Tong *et. al.* (1998), reported seven isolates of *Trichoderma viride* against *F. oxysporum* and these exhibited inhibition zones above 3 mm in paired cultures. The inhibition of *Fusarium* was 70.5 % and 88.5 % respectively and these also promoted soybean growth.

Mathew and Gupta (1998) reported *T. harzianum* was the most effective *in vitro* as a biological control agent of *R. solani* on soybean in green house. Hazarika and Das (1998) tested isolates of *T. harzianium, T. viride* and *T. virens* from Assam and Tamil Nadu, India for their potential to control *R. solani.* Both when applied as seed or soil treatments effectively controlled root rot of soybean.

Ishrat *et. al.,* (1999) reported the wheat bean inoculum of *Pseudomonas aeruginosa* strains significantly ($p<0.05$) controlled infection of *Macrophomina phaseolina, F. solani* and *R. solani* on cotton, soybean, sunflower and bean.

Arafa and Mohamed (1999) reported the addition of mature (high C:N ratio) and immature (low C:N ratio) plant residues of barley and sorghum to soil significantly reduced number of chlamydospore germination in soybean root rot caused by *Fusarium.*

Hassanein *et al.,* (2000) and Sen (2000) evaluated several bioagents against diseases as a means to increase forage production. Some bioagents (*Trichoderma harzianum, T. viride, Bacillus subtilis*) and mycostop (*Streptomyces grisioviridis*) significantly inhibited mycelial growth of *R. solani* and *F. oxysporum in vitro*.

Zheng and Sinclair (2000) reported *Bacillus megaterium* to be effective for control of soybean root rot by *R. solani.* Mikhaeel *et. al.,* (2002) reported fungus *Glomus aggregatum* (VAM) and *Bacillus subtilis* when evaluated individually or in combination with *Bradyrhizobium japonicum* for reducing root rot disease caused by *F. oxysporum* in soybean and *Bradyrhizobium japonicum* were found effective biocontrol agents. Chakraborty *et. al*., (2003) and Guo *et. al.,* (2003) reported *Trichoderma harzianum, Bradyrhizobium japonicum* and rhizobacteria BH1 (*Bacillus* spp.) isolated from soybean rhizosphere, to be effective biocontrol agents to control soybean root rot disease caused by *F. solani* and *R. solani in vitro.*

Wang *et. al.,* (2004) reported the radiometric assessment of tillage and biological seed treatment with *Bradyrhizobium japonicum* suppressed soybean root rot disease caused by *F. oxysporum, F. solani* and *R. solani*. Hashem (2004) reported the efficacy of antagonists (*T. harzianum, Epicoccum nigrum* and *Paecilomyces lilacinus)* as well as two organic compounds (Strom and F-760) for control of *Macrophomina phaseolina, R. solani* and *F. oxysporum in vitro.* Mahdy *et. al.,* (2006) reported *B. thuringiensis* and *T. harzianum* to be effective bio control agents for control of *Meloidogyne javanica* and *R. solani.*

Botanicals, particularly neem (*Azadirachta indica*) has shown good fungicidal potential against *R. solani* and *F. solani* and seed promising as eco-friendly fungicide (Bhatnagar, 1992 and Mariappan, 1998). Lakshmanan and Nair (1984) and Manibhushan *et. al.,* (1998) reported the neem leaf extracts reduced the viability of sclerotia and mycelium growth of *R. solani*.

Workers have tried to develop integrated disease management strategy with emphasis on integration of chemicals and biological control agents for effective management of crop diseases caused by *R. solani* and *F. solani.* Curl *et. al.,* (1977) observed a slightly additive benefit of adding PCNB (penta chloro nitrobenzene @ 1 and 10 mg/ m^2 soil) with *Trichoderma* spp. in sterilized soil. Similarly, *R. solani* in bean was successfully managed by integration of PCNB, successive planting and use of *Trichoderma* (Henis *et. al.,* 1978).

Lewis and papavizas (1980) developed an integrated management strategy by using deep ploughing, seed treatment with captafol and soil application of biocontrol agents (*Gliocladium* and *Trichoderma*) for suppression of *R. solani* in soybean field. Chet *et. al.,* (1982) and Cole and Zvenyika (1988) developed an integrated management of *R. solani* and *Sclerotium rolfsii* by combining soil fumigation (methyl bromide), triadimenol fungicide and application of *T. harzianum* to the root zone of soybean.

Laciocladium and Pieta (1994) reported that *Trichoderma* spp. and *Gliocladium* spp. (*T. koningii, T. viride, G. catenulatum* and *G. roseum*) *Penicillium vermiculatum* and *T. roseum* gave better disease control in soybean that was comparable with chemical control by carboxin+thiram in pathogen (*F. oxysporum, F. solani, R. solani* and *S. sclerotiorum*) added separately to the garden soil.

Vyas (1994) reported *Trichoderma viride* and *T. harzianum* together with carbendazim and *T. viride* with carbendazim were found effective for reduce soybean root rot.

Haque and Ghaffar (1995) reported the seed dressing of soybean with *B. japonicum* alone or mixed with different concentrations of the fungicides Benlate (benomyl), Dithane M-45 (mancozeb), Bavistin (carbendazim) and Vitavax (carboxin) showed significant ($p<0.001$) protection of roots from infection caused by *M. phaseolina, R. solani* and *Fusarium* spp.

Minuto *et. al.,* (1997) obtained good control of *R. solani* on basil by using a combination of *Trichoderma* spp. (10^6 c.f.u./ g soil) and a reduced dose of tolclofos-methyl (0.25 g a.i./ m^2).

Cardoso *et. al.,* (1997) evaluated the relative efficacy of 18 fungicides and 7 antagonists for the control of soybean root-rot (*R. solani* and *F. solani*), foot rot (*S. rolfsii*) and ashy stem-rot (*M. phaseolina*) of *P. vulgaris* in green house trials in artificially infested soil. Tolclofos-methyl and quintozene were the only fungicides that significantly controlled all diseases under green house conditions, while *Trichoderma* isolate T 12 was the only antagonist able to suppress both root-rot and and foot-rot of soybean.

Jenson *et. al.,* (2004) reported that reduced tillage or mould board ploughing and seed treatment with *Bacillus subtilis* and/or *Bradyrhizobium japonicum* reduced root-rot of soybean caused by *R. solani.* Gyanendra and Verma (2005) reported good compatibility of fungicides (captan, mancozeb, thiram, carbendazim, carboxin and copper oxychloride at 0.05, 0.1 and 0.15 %), neem products and biological control agents (*T. harizanum* and *T. viride)* in control of soybean root-rot by *F. solani.*

Xue *et. al.,* (2007) reported the effective control through seed treatment with *Bacillus pumilus* GB 34, together with fungicides Allegiance (metalaxyl), Vitalfo-280 (carboxin + thiram), Apron or Maxim 480FS (fludioxonil) for control of soybean root rot caused by *R. solani.*

From the above review, it is evident that location specific integrated management module is required for soybean root rot.

3. MATERIALS AND METHODS

The study entitled **" Integrated Management of Root Rot of soybean (*Glycine max* L.)"** was under taken at the Department of Plant Pathology, Rajasthan College of Agriculture (RCA), Udaipur during 2009-10. The details of techniques followed and the methods and materials used during the course of experiment are described below.

3.1 GLASSWARE

The corning make glassware used in experiment were cleaned by dilute solution of potassium dichromate ($K_2Cr_2O_7$ - 60g, conc. H_2SO_4 60ml, distilled water 1000ml) washed with teepol and finally by distilled water and were dried before use.

3.2 CHEMICALS

All chemicals used for experiment work were of 'Analar' quality of British Drug House (Pvt.) Limited, Mumbai, 'Proanalysis Quality of E. Merck Limited, Sarabhai Chemicals, Baroda, Central Drug House Pvt. Ltd., Mumbai, Himedia Laboratory Chemicals, S.D. Fine Chem. Ltd., Bosisar, Labo-chemic Industrial Co., Mumbai.

3.3 STERILIZATION

Distilled water and media, etc. were sterilized at 1.045 kg cm^{-2} (15 pounds per square inch) in autoclave for 20 min. Petri plates were sterilized in hot air oven at 180°C for 2 hours. Soil was sterilized in autoclave at 1.045 kg/cm^{2} (15 psi) for 1 hour on 3 consecutive days.

3.4 ISOLATION, PURIFICATION AND IDENTIFICATION OF THE PATHOGENS

Samples of root-rot of soybean were collected from diseased plant sample received from village Kolyari, Tehsil Jhadole, Udaipur. The pathogens were isolated on potato dextrose agar (PDA) medium. Small pieces (1-2mm) of diseased roots were cut, washed with sterilized water, surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 2-3 minutes followed by three to four washings with sterilized

distilled water and were transferred aseptically to 2 per cent PDA (Potato Dextrose Agar)slants. The plates were incubated in an incubator at 25 $\pm$ 1^0C for 7 days. Hyphae coming out from the bits were sub-cultured on the fresh PDA in Petri dishes. From these bits mostly cultures of *Rhizoctonia solani* and *Fusarium solani* were recovered. The culture of *Rhizoctonia* was purified by single hyphal-tip method and that of *Fusarium* by single spore method using a dummy objective. The cultures were identified by comparing the morphological and cultural characters described in standard refrences Mordue (1988) for *Rhizoctonia* and Booth (1971) for *Fusarium,* and were identified as *Rhizoctonia solani* and *Fusarium solani.*

3.5 ISOLATION OF THE BIOCONTROL AGENTS

Isolation of the biocontrol agents were attempted from rhizosphere soil of both healthy and diseased soybean plants. Specific techniques were employed for isolating mycoparasities and antagonistic microorganisms. The detailed method is as follows :

3.5.1 Isolation of biocontrol agents using selective media :

Isolation of fungal (*Trichoderma viride*) and bacterial biocontrol agents was attempted by using selective media by dilution plate method (Warcup, 1955). For *Trichoderma* potato dextrose agar amended with 25 ppm chloramphenicol and 2 ml Triton X- 100/litre (Budge and Whipps, 1991) was used. Since the amount of rhizosphere soil was limited one gram rhizosphere soil was used and soil dilution of 10^5 was proposed. One ml of soil dilution was pipetted in sterilized Petri dishes and on this 20 ml of molten almost cool medium was poured and the plates were gently rotated to uniformly spread the propagules in the plates and than allowed to solidify. The plates were incubated at 25^0C for 5 days and resultant *Trichoderma* colonies were examined under the stereobionocular microscope and picked up on fresh 0.1 per cent Malt extract Agar medium (MEA). Hypal tip pure cultures were made and the cultures were maintained on 0.1 per cent MEA. The multiplication of *Trichoderma* was grown on dextrose agar medium while for bacteria King`s B medium (King *et. al.*, 1954) was used.

3.6 PATHOGENECITY TEST

Pathogenecity of the isolated cultures of *F. solani* and *R. solani* was tested by growing soybean plants in pots containing pathogen-infested soil. The pathogens *R. solani* and *F. solani* were separately multiplied on corn meal-sand (1:1) medium at 25 $\pm$ 1^0C for 10 days and then each was mixed separately with sterilized soil @ 20 g/kg soil. This inoculated soil was filled in sterilized plastic pots (20cm face diameter). The pots filled with inoculated soil were kept in the green house for 7 days and were irrigated with sterile water to allow establishment of the pathogen. Surface sterilized seeds (0.1 per cent mercuric chloride solution for 2 minutes) of susceptible soybean cultivar `JS-335` were sown in inoculated pots @10 seeds / pot, keeping four pots as four replications for each pathogen. For comparison seeds were sown in sterilized soil, without pathogen (un-inoculated control). The pots were irrigated on alternate days with sterilized water to provide good moisture. The typical symptoms of root rot started on 4th day and fully manifested within 10 days. The collar region and roots showed black lesions and shrunk. From the wilted seedlings showing black lesions of roots, re-isolation of the pathogens (*R. solani* and *F. solani*) was attempted, and both the cultures were readily re-isolated. In checks, healthy soybean plants continued to grow and developed seeds. The cultures were purified and maintained on PDA slants at 4^0 C for further studies.

3.7 *IN VITRO* EVALUATION OF DIFFERENT CULTIVARS OF SOYBEAN FOR SEED TRANSMISSION OF PATHOGENS (*F. solani* and *R. solani*)

3.7.1 Using blotter method

Seed transmission of *F. solani* and *R. solani* was studied on five soybean cultivars viz., JS- 335, NRC- 76, JS 93-05, JS 97-52, JS 95-60. The sterilized plastic Petri plates of 90 cm lined with blotting paper on both the surfaces were used. Seeds of each cultivar were surface sterilized using 0.1 per cent mercuric chloride solution for 2 minutes followed by three washings with sterilized, distilled water and then dried. The Blotters lined in the petri plates were then moistened using sterilized distilled water. 10 seeds per plate were used keeping five replications for each cultivar standard untreated check for each cultivar was also maintained with five replications for comparison. The plates were incubated at 25 $\pm$ 1^0C for 7 days and blotters were aseptically moistened on alternate days

with sterilized distilled water to provide good moisture. Observations were recorded after seven days of incubation.

3.7.2 Using PDA (Potato Dextrose Agar) medium

Seed transmission of *F. solani* and *R. solani* in all the five soybean cultivars was also studied using PDA medium. 20 ml of sterilized melted PDA was aseptically poured in sterilized Petri plates and was allowed to solidify. Seeds of all the cultivars were surface sterilized (using 0.1 per cent mercuric chloride solution for 2 minutes followed by three washings with sterilized distilled water then dried). 10 seeds per plate were used keeping five replications for each cultivar. Standard untreated check for each cultivar was also maintained for comparison. The plates were incubated at 25 $\pm$ 1^0C for 7 days. Observations were recorded after seven days of incubation.

3.8 EVALUATION OF FUNGICIDES, BIOCONTROL AGENTS AND BOTANICALS AGAINST SOYBEAN ROOT ROT PATHOGENS (*F. solani* and *R. solani*) *IN VITRO*

3.8.1 *In vitro* efficacy of fungicides (Poison food technique)

Relative efficacy of different systemic and non-systemic fungicides was evaluated by using poisoned food technique (Schmitzs, 1930). Seven fungicides *viz.*, thiram 75% WP [tetramethyl thiuram disulphide (TMTD)] Gupta Chemicals (p.) Ltd., Mumbai, Vitavax 75% WP [5,6-Dihydro-2-Methyl-1,4-Oxathiin-3-Carboxamide (carboxin)] Pesticide India Ltd., Udaipur, Bavistin 50% WP [carbendazim, Methyl-2-benzimidazole carbamate (MBC)] BASF India Ltd., Mumbai, Hexaconazole 5% EC [2-(2,4-dichlorophenyl) -1- (1H-1,2,4-triazol-1-yl) hexan-2-ol], Crop Life Science Ltd., Gujrat, tebuconazole 25.9 w/w [1-(4-chlorophenyl) -4,4-dimethyl-3-(1,2,4-triazol-1-methyl) peptan-3-ol (Folicular 250 EC)] Bayer Crop Science, India Ltd., Mumbai, Dithane M-45 75% WP [mancozeb, Mangnese ethylene bis-dithiocarbamate + zinc ions 2%] Indofil Chemicals Ltd., Mumbai and Copper oxychloride 50% WP [Bitox-50, dicopper chloride

trioxide] United Phosphorus Ltd., Mumbai were tested at two concentrations *i.e.* 500 and 1000 ppm. Desired quantity of each fungicide was added separately to sterilized medium, mixed thoroughly and poured in sterilized Petri dishes and allowed to solidify. Each plate was inoculated with 2 mm disc of fungal culture and incubated at 28 ± 1^{0}C. The linear growth after 7 days was recorded and per cent inhibition was calculated according to Vincent's Formula (1947) as follows :

$$\text{Per cent inhibition} = \frac{(C - T)}{C} \times 100$$

Where,

C = Diameter of the colony in control.

T = Diameter of the colony in treatment.

A check was also maintained where medium was not supplemented with any fungicide.

3.8.2 *In vitro* evaluation of neem formulations (Poison food technique)

Efficacy of two neem based formulations viz., *Azadirachtin* (0.2%) and Neem oil (0.2%), developed by Godrej Agrovet Pvt. Ltd., Mumbai, for commercial purpose. Neem oil and *Azadirachtin* were tested against *R. solani* and *F. solani* by poison food method. The formulations were incorporated in PDA 0.2 per cent and PDA dispersed in sterilized 90 mm glass Petri plates. For comparison, plates having PDA without neem formulations were kept as control. For each treatment, five replications were taken. Two mm bits of *R. solani* or *F. solani* removed from the periphery of 7 - days - old actively growing cultures were aseptically inoculated in the centre of each plate. The plates were incubated at 28 ± 1 ^{0}C for 7 days and then colony diameters were measured and compared with control plates when the respective pathogens were grown on PDA without neem formulations.

3.8.3 *In vitro* efficacy of bio-control agents (Dual culture technique)

The efficacy of biocontrol agents *i.e*. *Trichoderma viride* was tested by using dual culture plate method on PDA medium (Johnson *et al.*, 1959). Antagonistic effect of *T.*

viride was tested against the test pathogen (*F. solani* and *R. solani*), 2 mm diameter mycelium bit of 7 - days - old culture of *F. solani* and *T. viride*, *R. solani* and *T. viride* were placed separately at some distance on the periphery of Petri dishes containing sterilized PDA medium. For each treatment five replications were taken. Inoculated plates were incubated at $28\pm1\ ^{0}C$ temperature in incubator. Linear growth of pathogens and zone of inhibition was measured after 7 days of inoculation. PDA plates inoculated with pathogen alone served as check.

3.8.4 *In vitro* efficacy of bacterial antagonists (Dual culture technique)

Dual culture method was used for assessing inhibition of radial growth of the pathogen by bacteria inoculated on King`s B agar medium in sterilized Petri dishes. A loopful from the 24 hr old cultures was streaked on two side of each plate and in the centre 2 mm disc of *F. solani* or of *R. solani*, were aseptically inoculated. Control plates were inoculated by only pathogens individually. Five replications were maintained for each treatment. The plates were inoculated at $28\pm1\ ^{0}C$. The measurement of radial growth of the pathogens were recorded after 5 days and compared with that in the respective controls.

3.9 EVALUATION OF BIOCONTROL AGENTS AND FUNGICIDES FOR SUPPRESSION OF ROOT ROT OF SOYBEAN *IN VIVO*

3.9.1 Multiplication of *F. solani* and *R. solani* for soil application

For the pot experiment culture of *F. solani* and *R. solani* were multiplied on autoclaved corn meal-sand (2:1) medium for seven days and then mixed with sterilized garden soil @ 100 g/kg soil and multiplied for 7 days. This inoculum soil mixture was placed on the top of non-sterilized soil + FYM mixture (3:1) in fresh earthen pot (30 cm face diameter) @ 20 g/pot. For comparison two types of control were kept. In one control, pots had inoculated soil and another set of control pots had sterilized soil without inoculation. For each treatment three pots as three replications were maintained. All the pots were lightly irrigated immediately after inoculation. The pots were kept in the cage house for five days to allow establishment of the pathogen before sowing.

3.9.2 Seed treatment with biocontrol agents, bacterial antagonists and fungicides

Fungicides and biocontrol agents found effective *in vitro* were evaluated individually and in combinations *viz*, carbendazim, tebuconazole, *T. viride*, bactria,

carbendazim + *T. viride*, tebuconazole + *T. viride,* carbendazim + bacteria, tebuconazole + bacteria, *T. viride* + bacteria, carbendazim + *T. viride* + bacteria and tebuconazole + *T. viride* + bacteria as seed treatment in pot experiment. For seed treatment, cultures of the biocontrol agents were individually grown on 2 per cent malt extract agar (MEA), while bacteria were grown on King`s B medium. The spores colonies so developed were harvested by suspending in 20 ml water in each Petri dish and mixed with sterilized fine clay (talc powder) 10 gm to make a slurry. These formulations of the individual BCA`s were used for seed treatments @ 6 g/kg seed. The coated seeds were kept overnight in moist chamber so as to enable the antagonists to establish on seeds.

3.9.3 Fungicidal seed treatment

Since as only small quantity of each fungicides was to be used for seed treatment, the seeds were soaked in carbendazim 50 WP @ 0.1 % solution or tebuconazole 29.5 EC @ 0.2 ml/ kg seed for 30 minutes. The treated seeds were air dried in shade and then sown.

$$\text{Percent root rot} = \frac{\text{Total number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

3.9.4 Sowing and inter-culture operations

The various treated seeds of soybean (var. NRC -76) were sown in pots @ 30 seeds / pot, keeping three replications for each treatment. Just before sowing, soil samples were taken from each pot at the depth of 2 inches to determine initial population densities of the two pathogens.

3.10 OBSERVATIONS

The numbersof total seedlings were recorded 10 days after sowing. The numbers of root rot infected plants were recorded from germination till 40 - 45 days after sowing or at the time of harvesting. The biomass (dry matter produce) of soybean were recorded of each replications of each treatments after harvesting at 60 days of sowing. The collected samples were air dried in shade at 24 hrs, and then kept in an oven at 60^0C for 24 hrs, then cooled to room temperature and weighted for the dry matter.

To determine the population of biocontrol agents and their possible effect on *R. solani* and *F. solani* soil samples from soybean rhizosphere and around from both diseased and healthy plants were collected from each pot of each treatment carefully by uprooting the plants and lightly shaking these to remove the extra soil. The rhizosphere soil was collected by lightly scrapping with a hard brush. Samples of all the three replications of each treatment were pooled and placed in polythene bags, labelled and brought to the laboratory. Sub-samples from these pooled samples were used for determining population densities of biocontrol agents and the pathogen as per the methods described below :

3.11 DETERMINATION OF POPULATION [C.F.U. (colony forming units)] OF THE BIOCONTROL AGENTS AND PATHOGENS

The population C.F.U. (colony forming units) of fungal biocontrol agents, bacteria and *F. solani / R. solani* were determined by dilution plating (Warcup, 1955) on organism specific media. For estimation from soil, 1g soil was suspended in 100ml water, vigorously shaken and further serially diluted. Dilution of 10^5 was used to determine population of fungi biocontrol agents and *F. solani* and 10^4 for *R. solani*. For *Trichoderma,* specific medium – modified PDA Triton X- 100 media (Budge and Whipps, 1991) was used. For *F. solani* / *R. solani,* peptone PCNB medium (Nash and Synder, 1962) and for bacterial biocontrol agents King`s B medium (King *et. al.,* 1954) was used. Details of the composition of media are given in Appendix XIV. One ml of soil dilution was pipetted in sterilized Petri plates and on it soil, and then medium was allowed to solidify. The plates were incubated at 28 ± 1 ^{0}C for 5 days and the colonies of the organisms were counted with the help of a colony counter. The populations were computed to get c.f.u. / seed and c.f.u. /g soil, respectively.

3.12 STATISTICAL ANALYSIS

The data were subjected to analysis of variance and least significant difference (critical deviation) determined at 5 and 1 per cent probability. Treatments means were compared using C.D. (critical difference) to determine efficacy of the different treatments (Appendix I to XIII).

4. EXPERIMENTAL RESULTS

4.1 ISOLATION AND IDENTIFICATION OF THE PATHOGEN :

The cultures of pathogens inciting root rot in soybean were isolated from the samples collected from diseased plants received from village Kolyari Tehsil Jhadol, Udaipur. From these samples, cultures of both *Rhizoctonia* (60%) and *Fusarium* (40%) were obtained.

Pathogenicity of the cultures of *Rhizoctonia* and *Fusarium* were tested on pot-grown soybean plants. The cultures were individually multiplied on corn meal-sand medium and mixed in autoclave sterilized soil @ 200g. Surface sterilized seeds [0.1 per cent (mercuric chloride) for 2 minutes] of soybean cultivar `JS -335` were sown @ 15 seeds / pot. Pots with un-inoculated sterilized soil was kept as control.

The symptoms of root-rot followed by mortality started appearing in 10 days and were recorded on 15th day after sowing. In *Rhizoctonia* inoculated pots, young seedlings (10 days old) started drying and by 15 days quite many seedlings had died (Plate-1). The browning was observed in the form of characteristic bands of discoloured and normal tissues, and also as sunken, brown girdles around collar portion. The roots also turned brown and dried. In case of *Fusarium,* collar porton of the seedlings turned discoloured, brown and dried (Plate 1). The leaves of the infected plants drooped and the whole plant dried. The infected stems had elongated sunken lesion.

Re-isolation from the diseased plants yielded the cultures of both the pathogens which were identical to the original ones inoculated in these pots. The cultures were studied for morphological characteristics and compared with the standard descriptions of Mordue (1988) for *Rhizoctonia* and Booth (1971) for *Fusarium,* these were identified as *Rhizoctonia solani* and *Fusarium solani.* The purified cultured cultures were maintained on PDA slants in a refrigerator at 4^0C.

4.2 ISOLATION OF BIOCONTROL AGENTS :

Isolation of the bio-control agents were done from the air dried rhizosphere soil of healthy soybean roots using selective media. The studies resulted in recovery of those organisms which are known to act as bio-control agents of plant pathogens. The isolated cultures of *Trichoderma* spp. and bacteria were identified as *Trichoderma viride* and *Bacillus* spp. (Gram^{-ive}), these were used for further *in vitro* screening (Plate 2).

4.3 *IN VITRO* EVALUATION OF DIFFERENT CULTIVARS OF SOYBEAN FOR SEED TRANSMISSION OF ROOT-ROT PATHOGENS (*Fusarium solani* and *Rhizoctonia solani*)

4.3.1 Using blotter method

The effect of root rot causing pathogens (*F. solani* and *R. solani*) was studied on five different cultivars of soybean. The surface sterilized and un-sterilized soybean seeds were aseptically kept on sterilized and wet blotter paper in petri dishes and observed after 7 days of incubation at 25 $\pm$ 1^0 C. The data revealed that both the pathogens *R. solani* and *F. solani* were recovered from almost all the test cultivars of soybean. The maximum recovery of *R. solani* on surface sterilized seeds was observed in NRC- 76 (14.1%), followed by JS- 335 (11.3%), JS 93-05 (10.9%), JS 97-52 (10.7%) and lowest on JS 95-60 (10.1%). Similarly maximum recovery of *R. solani* on unsterilized seeds was observed on *cv*. NRC- 76 (39.9%), followed by JS- 335 (32.8%), JS 93-05 (31.4%), JS 97-52 (25.0%) and lowest on JS 95-60 (23.1%), respectively (Table 1 and Plate 3).

On surface sterilized seeds the *F. solani* was recovered maximum in NRC- 76 (14.0%), followed by JS 97-52 (13.1%), JS- 335 (11.5%), JS 93-05 (10.7%) and lowest recovery was seen in *cv*. JS 95-60 (8.7%). Like wise maximum recovery of *F. solani* on unsterilized seeds was observed on NRC- 76 (39.5%), followed by JS 97-52 (25.9%), JS- 335 (24.0%), JS 93-05 (20.0%) and lowest recovery was observed in *cv*. JS 95-60 (15.5%), respectively (Table 2 and Plate 3).

In general the maximum recovered of both pathogens *R. solani* and *F. solani* was on *cv.* NRC- 76 from surface sterilized and unsterilized seeds. Lowest recovered of *R. solani* and *F. solani* was from *cv*. JS 95-60 (Table 1,2 and Plate 3).

3.3.2 Using PDA (Potato Dextrose Agar) medium

The seed borne nature of the two root rot causing pathogens (*F. solani* and *R. solani*) was studied on five different cultivars of soybean. The surface sterilized and un-sterilized soybean seeds were aseptically kept on PDA (Potato Dextrose Agar) medium in sterilized Petri dishes and observed after 7 days of incubation at 25 $\pm$ 1^0 C. The data revealed that both the pathogens *R. solani* and *F. solani* were recovered from almost all the test cultivars of soybean. The maximum recovery of *R. solani* on surface sterilized seeds was observed in NRC- 76 (14.9%), followed by JS- 335 (12.0%), JS 93-05 (10.9%), JS 97-52 (10.2%) and lowest from JS 95-60 (9.0%). Similarly maximum recovery of *R. solani* on unsterilized seeds was observed on *cv*. NRC- 76 (40.9%), followed by JS- 335 (37.9%), JS 93-05 (37.0%), JS 97-52 (31.2%) and lowest from JS 95-60 (27.4%), respectively (Table 1 and Plate 3).

On surface sterilized seeds the *F. solani* was recovered maximum in NRC- 76 (14.1%), followed by JS 97-52 (13.8%), JS- 335 (12.5%), JS 93-05 (11.8%) and lowest recovery was seen in *cv*. JS 95-60 (11.7%). Like wise maximum recovery of *F. solani* on unsterilized seeds was observed on NRC- 76 (33.4%), followed by JS 97-52 (30.2%), JS-335 (29.9%), JS 93-05 (29.1%) and lowest recovery was observed in cv. JS 95-60 (24.0%), respectively (Table 2 and Plate 3).

In general the maximum recovered of both pathogens *R. solani* and *F. solani* was on *cv*. NRC- 76 in surface sterilized and unsterilized seeds. Lowest recovered of *R. solani* and *F. solani* was on *cv*. JS 95-60 (Table 1,2 and Plate 3).

4.4 *IN VITRO* EVALUATION OF FUNGICIDES, BIOCONTROL AGENTS AND BOTANICALS AGAINST SOYBEAN ROOT ROT CAUSED BY *F. solani* AND *R. solani*

4.4.1 *In vitro* evaluation of fungicides (poisoned food technique)

Seven fungicides thiram, vitavax, carbendazim, hexaconazole, tebuconazole, mancozeb and copper oxychloride were evaluated at two concentrations *viz.,* 500 and 1000 ppm with poisoned food technique against *F. solani* and *R. solani*. All the test

fungicides significantly inhibited the mycelial growth of *F. solani* and *R. solani* at both concentrations.

The effect was more on *R. solani* where three fungicides carbendazim, mancozeb and hexaconazole completely inhibited the growth both at 500 and 1000 ppm concentrations. On the other hand only carbendazim could inhibit the growth of *F. solani* both at 500 and 1000 ppm concentrations. Further, the fungicides varied in their efficacy on the particular pathogen. Here Tebuconazole at 1000 ppm exhibited 100 per cent growth inhibition of *R. solani* and at 500 ppm per cent growth inhibition was 97.5 per cent. This was followed by vitavax which exhibited 97.7 per cent and 92.0 per cent growth inhibition at 1000 and 500 ppm, respectively. The other test fungicides copper oxychloride and thiram were found to show very weak efficacy to control of *R. solani* at both 500 and 1000 ppm (Table 3 and Plate 4). The per cent growth inhibition for thiram was 0.3 per cent each at 500 ppm and 1000 ppm and copper oxychloride the per cent growth inhibition was 0.2 per cent each for 500 as well as 1000 ppm as compared to control (Table 3 and Plate 4).

On the other hand two fungicides carbendazim and tebuconazole could inhibit the growth (100%) of *F. solani* both at 500 and 1000 ppm concentrations. These were followed by hexaconazole where per cent growth inhibition was 98.2 per cent and 97.9 per cent at 1000 and 500 ppm, respectively . Copper oxychloride, vitavax and mancozeb were found at par in inhibiting of *F. solani* at 1000 ppm, where per cent growth inhibition was 93.8 per cent, 92.9 per cent and 91.9 per cent respectively. At 500 ppm mancozeb (84.2 per cent) was found superior over vitavax (77.8 per cent) and copper oxychloride (64.9 per cent). Thiram was found to be the weakest fungicide both at 1000 (66.9 per cent) and 500 (58.3 per cent) as compared to the control (Table 4 and Plate 5).

4.4.2 ***In vitro* evaluation of botanicals (poisoned food technique)**

Two neem based formulations viz., neem oil and *Azadirachtin* were evaluated at 0.2 per cent concentration with poisoned food technique against *R. solani* and *F. solani. Azadirachtin* caused 19.9 per cent inhibition growth of *R. solani* and 25.3 per cent inhibition growth of *F. solani,* while neem oil was less effective, and caused 4.2 per cent inhibition of linear growth of *R. solani* and 6.5 per cent of *F. solani.* In general, both the

botanical were not much effective in this study and therefore these were not taken forward for further pot culture experiments (Table 5 and Plate 4).

4.4.3 *In vitro* evaluation of biocontrol agents (Dual culture technique)

Efficacy of biocontrol agents the local isolates of *T. viride* and bacteria (*Bacillus* spp.) as studied *in vitro* as described in Materials and Methods, using dual culture technique. Data revealed that *T. viride* and bacteria (*Bacillus* spp.) were potential antagonists of *R. solani* and *F. solani.*

Maximum and significant high per cent inhibition of growth (92.8 per cent) by bacteria was observed in dual culture method for *R. solani,* followed by *T. viride* (86.3 per cent), respectively. Efficacy of *T. viride* was found comparatively lower than bacteria (*Bacillus* spp.), (Table 6 and Plate 6).

The sensitivity towards another pathogen *F. solani* was less as compared to *R. solani.* The inhibition of growth of *F. solani* in dual culture method was higher by bacteria (86.8 per cent), as compared to *T. viride* 81.5 per cent, respectively (Table 7 and Plate 6).

4.5 *IN VIVO* EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS AS SEED TREATMENT AGAINST SOYBEAN ROOT ROT CAUSED BY *F. solani* AND *R. solani*

The fungicides and biocontrol agents found effective *in vitro*, were further evaluated as seed treatment for suppression of soybean root-rot in a pot experiment, with inoculation of both the pathogens. Cultures of *R. solani* and *F. solani* were multiplied separately on corn meal-sand medium in polypropylene bags at 28 ± 1^{0} C for 15 days till good growth and sporulation occurred. These were then mixed in equal amount and to this equal amount of sterilized soil was added. This inoculum was added on top of soil : FYM (3:1) mixture filled in earthen pots @ 200 g/ pot and properly mixed in the top 5' soil. The pots were lightly irrigated to provide congenial condition for pathogen to establish and multiply and were left as such for 5 days. Three pots as three replications were kept for each treatment. A replicated control without seed treatment was also maintained for comparison. At the time of sowing, soil samples were taken from a depth of 2.5" to determine initial population of *R. solani* and *F. solani.* Seeds of soybean

cultivar 'NRC- 76' (That showing maximum recovery of *F. solani* and *R. solani*) were variously treated with *Trichoderma viride,* bacteria (*Bacillus* spp.) or fungicides (carbendazim 0.1 per cent and Tebuconazole 0.2 per cent EC, those were found best *in vitro* studies), and their combination were planted in the respective pots @ 30 seeds / pot.

A light irrigation was given immediately after sowing. The pots were kept in the green house. Observations for germination were recorded 10 days after sowing and for root-rot and plant mortality after 10 days of sowing. After 45 days, the plants from each pot were up-rooted, soil samples from rhizosphere were collected for determination of population densities of the respective biocontrol agents and the two pathogens. The soil was then washed off from the roots and plants were at first air-dried for 24 hours and then dried in an oven at 60^0 C for three days and dry weight of biomass was determined for each replication in each treatment.

The population of biocontrol agents and *F. solani* were determined by dilution planting (10^5) and of *R. solani* (10^4) on selective media, as described in details in Materials and Methods. The results of this study are described as below: For comparison, the control where un-treated seeds were sown in the pathogen inoculated soil.

The soybean seeds germinated well in all the treatments, but there was reduction in germination in control (61.0 per cent) (Table 8). The differences in germination among these treatments and control were statistically (*P=0.05*) significant. All the treatments resulted in higher germination as comparison to control (Table 8 and Plate 7). Highest germination (89.8 per cent) was observed in seed treatment with carbendazim + *T. viride* + bacteria (*Bacillus* spp.). Closely followed by carbendazim + bacteria (86.7 per cent), tebuconazole + bacteria (85 per cent), carbendazim + *T. viride* (81.4 per cent), tebuconazole + *T. viride* (78.7 per cent), *T. viride* + bacteria (75 per cent), tebuconazole + *T. viride* + bacteria (71.6 per cent), after that the carbendazim, tebuconazole, bacteria and *Trichoderma viride* showed lower (65-70 per cent) per cent of germination as compared to other treatments. The germination in these treatments was significantly higher over control.

The disease symptoms started after 10 days and highest seedling mortality (49.5 per cent) was observed in control. Per cent mortality was significantly lower in all other

treatments as compared to control. Three treatments showing good and significant suppression of the disease 9.3, 9.6, 14.2 per cent mortality, were carbendazim + *T. viride* + bacteria(*Bacillus* spp.), carbendazim + bacteria (*Bacillus* spp.) and tebuconazole + bacteria (*Bacillus* spp.) respectively, followed by carbendazim + *T. viride* (21.2 per cent mortality), tebuconazole + *T. viride* (23.7 per cent mortality), *T. viride* + bacteria (26.7 per cent mortality), tebuconazole + *T. viride* + bacteria (28.5 per cent mortality) per cent mean mortality. While *Trichoderma viride* alone was the less effective (39.8 per cent mortality), as compared to carbendazim alone (29.5 per cent mortality), tebuconazole alone (35.6 per cent mortality) and bacteria (*Bacillus* spp.) alone (37.7 per cent) mortality in plants (Table 8 and Plate 7).

The seed treatments with carbendazim + *T. viride* + bacteria that showed lowest 9.3 per cent mortality and carbendazim + bacteria with 9.6 per cent mortality were found most effective as compared to 49.5 per cent mortality in the control and found at par in disease suppression. The differences in disease incidence among these three treatments were statistically significant which showed less disease incidence compared to control.

All the treatments resulted in significantly higher mean dry weight of the plants as compared to the un-treated control (2.6g). The highest dry weight was recorded in carbendazim + *T. viride* + bacteria (7.2g) and carbendazim + bacteria (6.1g) followed by tebuconazole + bacteria (5.4), carbendazim + bacteria (5.1g), *T. viride* + tebuconazole (4.7g), *T. viride* + bacteria (4.4g) and tebuconazole + *T. viride* + bacteria (4.0g), dry weight with seed treatment alone carbendazim, tebuconazole and bacteria was found to be 3.7, 3.2 and 3.1g respectively, while the least dry weight was observed in *Trichoderma viride* (3.0g), (Table 8). In general the superior seed treatment of soybean seeds against root rot caused by *R. solani* and *F. solani* was seed treatment with carbendazim + *T. viride* + bacteria (*Bacillus* spp.), followed by carbendazim + bacteria and tebuconazle + bacteria (*Bacillus* spp.) as second and third best treatment for control of disease incidence and increased in germination per cent and mean dry weight (Table 8 and Plate 7).

4.6 POPULATION DENSITIES OF THE BIOCONTROL AGENTS AND THE PATHOGENS IN DIFFERENT TREATMENTS :

These biocontrol agents could successfully establish in the soybean rhizosphere and multiplication to reach high densities. These BCAs varied in their ability to establish and proliferation in soybean rhizosphere. Among the *T. viride* treatments highest population counts (c.f.u. / g soil) in the rhizosphere were of seed treatment with carbendazim + *T. viride* + bacteria ($9.0x10^5$), followed by *T. viride* ($8.6x10^5$), tebuconazole + *T. viride* + bacteria ($8.0x10^5$), *T. viride* + bacteria ($7.8x10^5$) and tebuconazole + *T. viride* ($6.9x10^5$), while Carbendazim + *T. viride* exhibited lowest ($6.7x10^5$ / g soil) population counts as compared to other treatments (Table 9 and plate 8).

Similarly, population density of soybean rhizosphere bacteria (*Bacillus* spp., G^{-ive}) in different treatments as individual or/and in combination. The highest population counts of bacteria in the rhizosphere were exhibited by seed treatment with tebuconazole + bacteria ($9.9x10^5$), followed by carbendazim + bacteria ($9.6x10^5$), tebuconazole + *T. viride* + bacteria ($9.1x10^5$), bacteria alone ($8.9x10^5$) and carbendazim + *T. viride* + bacteria ($8.9x10^5$), while seed treatment with *T. viride* + bacteria exhibited lowest $8.6x10^5$ c.f.u. / g soil, population counts as compared to other treatments (Table 9 and Plate 8).

The population of *R. solani* (c.f.u. /g soil) in the rhizosphere was significantly higher in un-treated control, where c.f.u. / g soil of *R. solani* was $4.6x10^4$ c.f.u. / g soil. This population of *R. solani* was at par in the seed treatment with fungicides carbendazim alone ($2.5x10^4$) and Tebuconazole alone ($2.7x10^4$) c.f.u. /g soil, respectively (Table 9).

Among the BCAs seed treatments, the suppression of population of *R. solani* was maximum in carbendazim + *T. viride* + bacteria ($2.3x10^4$), followed by seed treatment with carbendazim + bacteria ($2.4x10^4$ c.f.u. / g soil), tebuconazole + *T. viride* + bacteria ($2.8\ x10^4$), *T. viride* ($3.2\ x10^4$), bacteria ($3.2x10^4$), tebuconzole + bacteria ($3.6\ x10^4$), carbendazim + *T. viride* ($3.8\ x10^4$) and *T. viride* + bacteria ($4.0\ x10^4$) and the lowest inhibition was exhibited by tebuconazole + *T. viride* ($4.3\ x10^4$) c.f.u. / g soil (Table 9).

Likewise *R. solani* the population density of *F. solani* was significantly suppressed due to different treatments tested in this study as compared to untreated control. The population density of *F. solani* after 45 days of sowing was $6.6x10^5$ / g soil in untreated control. The c.f.u. /g soil in seed treatment with fungicides carbendazim

alone and tebuconazole alone was found to be at par in 5.4 and $5.7x10^5$ / g soil, respectively. The lowest population density of *F. solani* was observed in treatment of carbendazim + *T. viride* + bacteria ($3.3x10^5$), followed by that in tebuconazole + *T. viride* + bacteria ($4.2x10^5$), *T. viride* ($5.0x10^5$), tebuconazole +*T. viride* ($5.0x10^5$), bacteria ($5.1x10^5$), tebuconazole + bacteria ($5.3x10^5$), *T. viride* + bacteria ($5.6x10^5$), carbendazim + bacteria ($5.9x10^5$), and while maximum population density of *F. solani* in seed treatment of carbendazim + *T. viride* 6.0 $x10^5$ / g soil as compared to other treatments, respectively (Table 9 and Plate 8).

In all the seed treatment of *T. viride* + bacteria + carbendazim in combination showed more suppression of *R. solani* and *F. solani* followed by carbendazim + bacteria and *T. viride* + bacteria (Table 9and Table 8).

5. DISCUSSION

The present investigation were undertaken with an aim to develop an effective strategy for the integrated management of root-rot of soybean (*Glycine max*), which is wide spread in all the soybean growing areas. The popular variety 'JS- 335' was found to have moderate to high infection in and around Udaipur. Since in the last few years, there has been a boost in soybean cultivation due to the economic value of soybean products like soybean oil, soyamilk etc., a strategy was felt necessary for its sustainable control of soybean root rot.

The symptoms of this disease were characteristic of root-rot and soybean plant of all growth stages were observed to be infected with it. Attempts to isolate pathogens from the diseased stems and roots, yielded culture of *Rhizoctonia solani* (60%) and *Fusarium solani* (40%). In several cases, from same pieces of infected roots or stems, both the cultures were recovered. The hyphae of *Rhizoctonia* grew more vigorously and spread away from that of *Fusarium* and it was easy to separate the two pathogens and identify. The *Rhizoctonia* induced damping-off type symptoms in the young seedlings, within a week, while *Fusarium* symptoms which started as browning of stem and slight yellowing of the leaves, were fully manifested in 15 days. The cultures were re-isolated from the diseased plants and compared with standard descriptions (Mordue, 1988 for *Rhizoctonia* and Booth, 1971 for *Fusarium*) and identified as *R. solani* and *F. solani.* The popular cultivar 'NRC- 76' yielded the maximum recovery of both the pathogens and considered the most suitable for pot studies.

A perusal of the literature revealed that although soybean root-rot caused by *R. solani* and *F. solani* has been reported by many, no comprehensive attempts have been made to develop effective management of this disease, despite its commercial value.

Though no specific information about perpetuation of these two pathogens in soybean is available but these are generally known to be seed as well as soil-borne in crops like chilli (Holliday, 1981; Surekha *et al*., 1986). The need was therefore felt to evaluate the seed transmission of *R. solani* and *F. solani* from the seeds of five popular

cultivars of soybean viz., JS- 335, NRC- 76, JS 93-05, JS 97-52 and JS 95-60 using blotter method and on PDA (Potato Dextrose Agar). It was found that maximum recovery of both the pathogens was from cultivar 'NRC- 76' and cultivar 'JS 95-60' exhibited lowest recovery of pathogens. It was also found in this study that there was some differential results in recovery of *R. solani* and *F. solani* from seeds of *cv*. JS- 335 and *cv*. JS 97-52. The recovery of *R. solani* from *cv*. JS 97-52 was less as compared to that from *cv*. JS- 335, where as it was vice versa for *F. solani,* where its recovery was less in *cv*. JS-335 as compared to that from *cv*. JS 97-52. Further, the experiment was conducted both on surface sterilized and un-sterilized seeds, suggesting external as well as internal seed borne nature of both the pathogen.

Since soybean root rot occurs on plants of all the growth stages, in the fields, need was felt for an effective integrated management of the disease and the pathogen had to be suppressed both in seed and soil. In view of increasing disease incidence, in popular soybean growing areas, attempts were made to evaluate seven fungicides at 500 and 1000 ppm concentrations (systemic and non systemic) and two neem based formulations (*Azadirachtin* and neem oil) at 0.2 per cent concentration against *R. solani* and *F. solani in vitro.* Sensitivity of carbendazim, mancozeb and hexaconazole was more pronounced against *R. solani,* where as two fungicides carbendazim and tebuconazole were highly effective against *F. solani* at both the concentrations. So the most effective ones carbendazim and tebuconazole were taken for further pot culture studies. Similar results have been reported by Wahid *et al.,* (1995), where bavistin and benlate at 100 ppm and vitavax at 500 ppm gave complete inhibition of soybean root-rot. Vrataric *et. al.,* (2002), suggested that seed treatment and foliar spraying of carbendazim and benlate for control of fungi causing soybean root-rot. Seed treatment of fungicides carbendazim, thiram and captan was also reported to the effective control of soybean root-rot caused by *R. solani* (Wang *et. al.,* 2004 and Taya *et. al.,* 1990).

Two neem based formulations (Neem oil 0.2% and *Azadirachtin* 0.2%), were found to be less effective against both the pathogen, so they were excluded from the further pot culture studies.

It was thought proper to evaluate efficiency of some biocontrol agents for suppression of these pathogens. For this attempts were made to isolate local isolates and could recover of *Trichoderma viride* and bacteria (*Bacillus* spp., G^{-ive}). *In vitro* studies for evaluating revealed the efficacy of both the isolates *T. viride* & bacteria in dual culture method. This was expected as in dual culture, all the modes of antagonism, competition as well as mycoparasitism are simultaneously operative. In the present study both the isolates of *T. viride* and bacteria were found to be highly effective against both the pathogens *in vitro*, however bacterial (*Bacillus* spp.) isolate was found to be superior over *T. viride* isolate. Similar results have been observed by several workers, where biological control agents like *T. harzianum, Gliocladium virens, Bacillus subtilis* and *Streptomyces* spp., have been reported to be effective for control of soybean root-rot pathogens (*R. solani, F. oxysporum* and *F. solani*), (Haque *et. al.,* 1990; Mousa and Mousa, 1994; Mousa, 1996; Hassanein *et al.,* 2000 and Sen, 2000).

The fungicides – carbendazim and tebuconazole and biocontrol agents – *Trichoderma viride* and bacteria (*Bacillus* spp., G^{-ive}) found effective *in vitro*, were further evaluated as seed treatment individually and in their combinations for suppression of soybean root-rot in a pot experiment,

The pot experiment conducted on 'NRC- 76' under mixed inoculation of *R. solani* and *F. solani* revealed minimum percent root rot (9.3%), with maximum germination per cent (89.8%) and maximum dry weight (7.2g), in the seed treatment with the carbendazim + *T. viride* + bacteria (*Bacillus* spp.). This was found at par with integration of seed treatment with carbendazim + bacteria (*Bacillus* spp.), where per cent root-rot (9.6%), germination per cent (86.7%), and dry weight of plants (6.1g), as compared to control with maximum (49.5%) per cent root-rot, minimum germination per cent (61.0%) and lowest dry weight of plants (2.6g). Seed treatment alone with the two fungicides and two BCAs although were superior over control but were lesser effective. Some similar results on integration of fungicides with BCAs have been observed by Vyas (1994), who reported *T. viride* and *T. harzianum* with carbendazim were found effective for reduction of soybean root-rot. Gyanendra and Verma (2005), reported good compatibility of fungicides carbendazim, neem products and biocontrol agents (*T. harzianum* and *T. viride*), for control of soybean root-rot.

The disease suppression in these treatments seemed to be due to reduction of the inoculum density of the pathogens. The bio-control agents effectively established in soybean rhizosphere and reached high population densities, while the population of the pathogens were low in the rhizosphere.

These BCAs are natural soil inhibitants and once established in the rhizosphere, these are able to suppress the pathogen by active antagonism, mainly antibiosis and competition (Chet, 1989 and Fravel *et. al.,* 1985) and also enhance plant growth by production of volatiles (Chang *et. al.,* 1986). Of the different methods of application of fungicides and biocontrol agents, seed treatments have been most favoured and used, and there are several studies to show that the BCAs applied on seed can establish in the rhizosphere and provide good suppression of the pathogens and diseases (De and Mukhopadhyay, 1990; Vyas, 2001 and Xue *et. al.,* 2007). The seed treatment with integration of fungicides with BCAs were found effective in the present study also this can be useful for practical control of root-rot of soybean.

In vitro studies revealed the efficacy of these BCAs against *R. solani* and *F. solani,* and *in vivo* studies integration of these BCAs resulted in noticeable suppression of both the pathogens. This information is useful to manage complex diseases like the present one and can be used for other complex root-rot disease caused by pathogens in crops like gram and ground nut.

Now there is need to study the efficacy of these integrated treatments for effective and sustainable management package for soybean root-rot. Need is also evaluate compatibility of bacterial isolate (*Bacillus* spp.) with *Trichoderma,* as has been reported by Mahdy *et. al.,* (2006), for developing a more comprehensive integrated management for soybean root-rot.

Table 4: *In vitro* efficacy of various systemic and non systemic fungicides against *Fusarium solani* after 7 days at 28 ± 2^0C (poisoned food technique)

Treatments	Colony diameter (mm)*		Per cent growth inhibition*	
	500 ppm	1000 ppm	500 ppm	1000 ppm
Carbendazim	0.0	0.0	100 (90.0)	100 (90.0)
Mancozeb	12.8	6.6	84.2 (66.6)	91.9 (73.5)
Hexaconazole	1.7	1.4	97.9 (81.7)	98.2 (82.3)
Tebuconazole	0.0	0.0	100 (90.0)	100 (90.0)
Vitavax	18.0	5.8	77.8 (61.9)	92.9 (74.5)
Copper oxychloride	28.5	5.1	64.9 (53.6)	93.8 (75.5)
Thiram	33.8	26.8	58.3 (49.8)	66.9 (54.9)
Control	81.0	81.0	0.0	0.0

	SEm ±	CD at 5%	CD at 1%	SEm ±	CD at 5%	CD at 1%
Fungicide	0.141	0.400	0.531	0.175	0.493	0.656
Concentration	0.093	0.262	0.348	0.114	0.323	0.429
F X C	0.245	0.692	0.920	0.302	0.854	1.135

* Mean of five replications. Figures in parentheses are arcsine √ per cent angular transformed values.

Table 3: *In vitro* efficacy of various systemic and non systemic fungicides against *Rhizoctonia solani* after 7 days at 28 $\pm$ 2^{0}C (poisoned food technique)

Treatments	Colony diameter (mm)*		Per cent growth inhibition*	
	500 ppm	1000 ppm	500 ppm	1000 ppm
Carbendazim	0.0	0.0	100 (90.0)	100 (90.0)
Mancozeb	0.0	0.0	100 (90.0)	100 (90.0)
Hexaconazole	0.0	0.0	100 (90.0)	100 (90.0)
Tebuconazole	2.0	0.0	97.5 (80.9)	100 (90.0)
Vitavax	6.5	1.9	92.0 (73.6)	97.7 (81.3)
Copper oxychloride	81.0	81.0	0.2 (2.5)	0.2 (2.5)
Thiram	80.9	80.9	0.3 (3.2)	0.3 (3.0)
Control	81.2	81.2	0.0	0.0

	SEm $\pm$	CD at 5%	CD at 1%	SEm $\pm$	CD at 5%	CD at 1%
Fungicide	0.015	0.042	0.055	0.018	0.051	0.068
Concentration	0.010	0.027	0.036	0.012	0.034	0.045
F X C	0.026	0.072	0.096	0.032	0.089	0.118

* Mean of five replications. Figures in parentheses are arcsine √ per cent angular transformed values.

Table 5: *In vitro* evaluation of botanicals for soybean root rot pathogen *Rhizoctonia solani* and *Fusarium solani* at 28 ± 2^0C (poisoned food technique)

S. No.	Treatments	*Rhizoctonia solani*		*Fusarium solani*	
		Colony diameter (mm)*	**Per cent Growth inhibition ***	**Colony diameter (mm)***	**Per cent Growth inhibition ***
1	Neem oil (0.2%)	78.5	4.2 (11.8)	74.8	6.5 (14.8)
2	*Azadirachtin* (0.2%)	65.6	19.9 (26.5)	59.8	25.3 (30.2)
3	Control	81.9	0.0	80.0	0.0
	SEm ±	1.415	0.203	1.545	0.265
	CD at 5%	4.360	0.625	4.761	0.817
	CD at 1%	6.112	0.876	6.674	1.146

* Average of five replications. Figures in parenthesis are arcsine √ per cent transformed values.

Table 6: *In vitro* evaluation of *Trichoderma viride* and Bacteria for antagonism to soybean root rot pathogen *Rhizoctonia solani* at 28 ± 2^{0}C (Dual culture technique)

S. No.	Treatments	Colony diameter (mm)* of *R. solani*	Per cent Growth inhibition * of *R. solani*
1	*R. solani* and *T. viride*	11.1	86.3 (68.3)
2	*R. solani* and Bacteria (*Bacillus* spp.)	5.8	92.8 (74.4)
3	Control	80.5	0.0
	SEm ±	0.164	0.184
	CD at 5%	0.506	0.568
	CD at 1%	0.709	0.796

* Average of five replications. Figures in parenthesis are arcsine √ per cent transformed values.

Table 7: *In vitro* evaluation of *Trichoderma viride* and Bacteria for antagonism to soybean root rot pathogen *Fusarium solani* at 28 ± 2^0C (Dual culture technique)

S. No.	Treatments	Colony diameter (mm)* of *F. solani*	Per cent Growth inhibition * of *F. solani*
1	*F. solani* and *T. viride*	15.0	81.5 (64.5)
2	*F. solani* and Bacteria (*Bacillus* spp.)	10.7	86.8 (68.7)
3	Control	81.1	0.0
	SEm ±	0.099	0.129
	CD at 5%	0.305	0.399
	CD at 1%	0.427	0.559

* Average of five replications. Figures in parenthesis are arcsine √ per cent transformed values.

Table 8: Effect of seed treatments with biocontrol agents and fungicides on per cent germination, per cent root rot and biomass production in soybean cultivar `NRC-76` in pathogen inoculated soil

S. No.	Treatments	Germination per cent	Per cent root rot	Dry weight of plants (g) (60 daya after sowing)
1	Carbendazim	70.8 (57.3)	29.5 (32.9)	3.7
2	Tebuconazole	67.7 (55.4)	35.6 (36.6)	3.2
3	Bacteria (*Bacillus* spp.)	67.0 (55.0)	37.7 (37.9)	3.1
4	*Trichoderma viride*	65.5 (54.1)	39.8 (39.1)	3.0
5	Carbendazim + *T. viride*	81.4 (64.6)	21.2 (27.4)	5.1
6	Tebuconazole + *T. viride*	78.7 (62.7)	23.7 (29.1)	4.7
7	Carbendazim + Bacteria (*Bacillus* spp.)	86.7 (68.6)	9.6 (18.1)	6.1
8	Tebuconazole + Bacteria (*Bacillus* spp.)	85.0 (67.8)	14.2 (22.1)	5.4
9	*T.* viride + Bacteria (*Bacillus* spp.)	75.0 (60.2)	26.7 (31.1)	4.4
10	Carbendazim + *T. viride* + Bacteria (*Bacillus* spp.)	89.8 (73.2)	9.3 (17.8)	7.2
11	Tebuconazole + *T. viride* + Bacteria (*Bacillus* spp.)	71.6 (57.9)	28.5 (32.3)	4.0
12	Control	61.0 (51.4)	49.5 (44.7)	2.6
	SEm ±	2.037	0.766	0.228
	CD at 5%	5.844	2.237	0.667
	CD at 1%	7.836	3.031	0.904

*Average of three replications. Figures in parentheses are arcsine √ per cent angular transformed values.

Table 2: Seed transmission of *F. solani* in different cultivars of soybean after 7 days of incubation at 25 ± 1^{0}C

S. No.	Soybean Cultivars	Blotter Method (Per cent recovery on seeds)		PDA (Potato Dextrose Agar) (Per cent recovery on seeds)	
		Surface sterilized seeds	Unsterilized seeds	Surface sterilized seeds	Unsterilized seeds
1	JS- 335	11.5 (19.8)	24.0 (29.3)	12.5 (20.6)	29.9 (33.2)
2	NRC- 76	14.0 (22.0)	39.5 (39.0)	14.1 (22.1)	33.4 (35.3)
3	JS 93-05	10.7 (19.1)	20.0 (26.6)	11.8 (20.1)	29.1 (32. 6)
4	JS 97-52	13.1 (21.1)	25.9 (30.6)	13.8 (21.8)	30.2 (33.3)
5	JS 95-60	8.7 (17.1)	15.5 (23.2)	11.7 (19.9)	24.0 (29.3)
	SEm ±	0.526	0.530	0.685	1.018
	CD at 5%	1.551	1.563	2.020	3.004
	CD at 1%	2.116	2.132	2.755	4.097

*Average of five replications. Figures in parentheses are arcsine √ per cent angular transformed values.

Table 1: Seed transmission of *R. solani* in different cultivars of soybean after 7 days of incubation at 25 ± 1^0C

S. No.	Soybean Cultivars	Blotter Method (Per cent recovery on seeds)		PDA (Potato Dextrose Agar) (Per cent recovery on seeds)	
		Surface sterilized seeds	Unsterilized seeds	Surface sterilized seeds	Unsterilized seeds
1	JS- 335	11.3 (19.5)	32.8 (34.9)	12.0 (20.2)	37.9 (38.0)
2	NRC- 76	14.1 (22.1)	39.9 (39.2)	14.9 (22.6)	40.9 (39.7)
3	JS 93-05	10.9 (19.2)	31.4 (34.1)	10.9 (19.2)	37.0 (37.5)
4	JS 97-52	10.7 (19.1)	25.0 (30.0)	10.2 (18.6)	31.2 (33.9)
5	JS 95-60	10.1 (18.5)	23.1 (28.7)	9.0 (17.3)	27.4 (31.5)
	SEm ±	0.519	0.486	0.967	1.414
	CD at 5%	1.532	1.433	2.852	4.172
	CD at 1%	2.089	1.955	3.890	5.691

*Average of five replications. Figures in parentheses are arcsine √ per cent angular transformed values.

Table 9: Population densities of biocontrol agents and pathogens in rhizosphere of soybean cultivar `NRC-76` 45 days after sowing

S. No	Treatments	Rhizosphere population of biocontrol agents and pathogens*			
		BCAs c.f.u./ g soil		*Rhizoctonia solani* sclerotia X 10^4 / g soil a	*Fusarium solani* c.f.u. X 10^5 / g soil
		Trichoderm viride X 10^5 / g soil	Bacteria X 10^5 / g soil		
1	Carbendazim	-	-	2.5	5.4
2	Tebuconazole	-	-	2.7	5.7
3	Bacteria (*Bacillus* spp., G^{-ive})	-	8.9	3.2	5.1
4	*Trichoderma viride*	8.6	-	3.2	5.0
5	Carbendazim + *T. viride*	6.7	-	3.8	6.0
6	Tebuconazole + *T. viride*	6.9	-	4.3	5.0
7	Carbendazim + Bacteria (*Bacillus* spp.)	-	9.6	2.4	5.9
8	Tebuconazole + Bacteria (*Bacillus* spp.)	-	9.9	3.6	5.3
9	*T.* viride + Bacteria (*Bacillus* spp.)	7.8	8.6	4.0	5.6
10	Carbendazim + *T. viride* + Bacteria (*Bacillus* spp.)	9.0	8.9	2.3	3.3
11	Tebuconazole + *T. viride* + Bacteria (*Bacillus* spp.)	8.0	9.1	2.8	4.2
12	Control	-	-	4.6	6.6
	SEm ±	0.151	0.168	0.098	0.159
	CD at 5%	0.442	0.491	0.286	0.465
	CD at 1%	0.599	0.665	0.387	0.630

* Average of three replications.
* Initial population of *R. solani* $1.5x10^3$ c.f.u. / g soil.
* Initial population of *F. solani* $3.5x10^3$ c.f.u. / g soil.

6. SUMMARY

The present study was undertaken on integrated management of root-rot of soybean, which is wide spread in all the soybean growing areas. The objectives were to determine relative pathogenic potential of *Rhizoctonia solani* and *Fusarium solani*, efficacy of bio-control agents, neem formulations and fungicides for suppression of *R. solani* and *F. solani in vitro*; and to evaluate bio-control agents, neem formulations and fungicides individually as well as in combinations for suppression of soybean root-rot in pot culture.

The disease was recorded on popular cultivar 'JS– 335' with moderate to high infection soybean fields around Udaipur. The cultures of pathogens inciting the root-rot of soybean were isolated from the samples collected from the diseased plants received from Kolyari, Tehsil Jhadole , Udaipur. From these samples cultures of both *Rhizoctonia* (60%) and *Fusarium* (40%) were obtained, and were identified as *Rhizoctonia solani* and *Fusarium solani*, and further their pathogenicity was confirmed.

The seed transmission of *R. solani* and *F. solani,* was studied in five popular cultivars *viz.*, JS- 335, NRC- 76, JS 93-05, JS 97-52 and JS 95-60 using Blotter and on Agar Plate method. Maximum recovery of both pathogens was from seeds of cultivar 'NRC- 76', while there from cultivar 'JS 95-60' exhibited lowest recovery of pathogens. There was differential results in recovery of *R. solani* and *F. solani,* from seeds of *cv*. JS- 335 and *cv*. JS 97-52. The recovery of *R. solani* from *cv*. JS 97-52 was less as compared to *cv*. JS- 335, where as it was *vice versa* for *F. solani*. Recovery of both the pathogen from both sterilized and un-sterilized seeds of all test cultivars indicates external as well as internal seed borne nature of both the pathogens.

In view of increasing disease incidence in soybean growing areas, attempts were made to evaluate seven fungicides at 500 and 1000 ppm concentrations (systemic and non systemic) and two botanicals (neem oil and *Azadirachtin*) at 0.2 per cent concentration against *R. solani* and *F. solani in vitro.* Sensitivity of carbendazim, mancozeb and hexaconazole was more pronounced against *R. solani,*

where as two fungicides carbendazim and tebuconazole were highly effective against *F. solani* at both the concentrations. Therefore, the effective fungicides carbendazim and tebuconazole were taken for further pot culture studies.

Both the neem based formulations (Neem oil 0.2% and *Azadirachtin* 0.2%), were less effective against both the pathogen, hence were not tested further.

In vitro studies for evaluating comparative efficacy and mode of antagonism to local isolates of BCAs, *T. viride* and bacteria (*Bacillus* spp., G^{-ive}) recovered from soybean rhizosphere were used. The efficacy of both the isolates found to be highly effective against both the pathogens *in vitro*. However, bacterial isolate (*Bacillus* spp.) was found to be more effective than *T. viride*.

The fungicides and BCAs, found effective *in vitro,* were further evaluated as seed treatment for suppression of soybean root-rot in pot experiment with pathogen inoculated soil. The seeds of popular soybean cultivar 'NRC- 76' that had shown maximum infection of *R. solani* and *F. solani,* were used in this experiment. Minimum per cent root rot (9.3 per cent), with maximum germination percent (89.8 per cent) and maximum dry weight (7.2g) were observed in seed treatment with carbendazim + *T. viride* + bacteria (*Bacillus* spp.) as compared to control with maximum (49.5 per cent) root-rot, minimum germination (61.0 per cent) and lowest dry weight (2.6g) integration of fungicides with both the BCAs was better effective over their individual applications as well as over the untreated control.

The disease suppression in these treatments could be correlated with reduction of the inoculum density of the pathogens. The test fungicides, BCAs and their integration significantly suppressed the population of the pathogens as compared to control. The BCAs effectively established in soybean rhizosphere and reached high population densities, while the population of the pathogens were low in rhizosphere.

Integration of fungicides and BCAs is not only economical but eco-friendly strategy for better control of this disease. These need to be further tested in micro plots for confirmation and recommendation for practical disease control in the farmer's field.

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*** APPENDICES ***

APPENDIX- I

ANALYSIS OF VARIANCE FOR SEED TRANSMISSION OF *R. solani* IN DIFFERENT CULTIVARS OF SOYBEAN AFTER 7 DAYS OF INCUBATION AT 25 ± 1^{0}C (TABLE-1)

Source of variance	d.f.	Blotter Method			
		Surface sterilized seeds		Unsterilized seeds	
		SS	MSS	SS	MSS
Treatment	4	50.88	12.72	71.69	17.92
Error	20	26.96	1.35	21.31	1.07

APPENDIX- II

ANALYSIS OF VARIANCE FOR SEED TRANSMISSION OF *R. solani* IN DIFFERENT CULTIVARS OF SOYBEAN AFTER 7 DAYS OF INCUBATION AT 25 ± 1^{0}C (TABLE-1)

Source of variance	d.f.	PDA (Potato Dextrose Agar)			
		Surface sterilized seeds		Unsterilized seeds	
		SS	MSS	SS	MSS
Treatment	4	26.04	6.51	230.28	57.57
Error	20	46.89	2.34	103.67	5.18

APPENDIX- III

ANALYSIS OF VARIANCE FOR SEED TRANSMISSION OF *F. solani* IN DIFFERENT CULTIVARS OF SOYBEAN AFTER 7 DAYS OF INCUBATION AT 25 ± 1^{0}C (TABLE-2)

Source of variance	d.f.	Blotter Method			
		Surface sterilized seeds		Unsterilized seeds	
		SS	MSS	SS	MSS
Treatment	4	86.77	21.69	1639.02	409.75
Error	20	27.66	1.38	28.07	1.40

APPENDIX- IV

ANALYSIS OF VARIANCE FOR SEED TRANSMISSION OF *F. solani* IN DIFFERENT CULTIVARS OF SOYBEAN AFTER 7 DAYS OF INCUBATION AT 25 ± 1^{0}C (TABLE-2)

Source of variance	d.f.	PDA (Potato Dextrose Agar)			
		Surface sterilized seeds		Unsterilized seeds	
		SS	MSS	SS	MSS
Treatment	4	99.78	24.94	595.36	148.84
Error	20	93.46	4.67	200.03	10.00

APPENDIX- V

ANALYSIS OF VARIANCE FOR *IN VITRO* EFFICACY OF VARIOUS SYSTEMIC AND NON SYSTEMIC FUNGICIDES AGAINST *Rhizoctonia solani* AFTER 7 DAYS AT 28 ± 2^{0}C (POISONED FOOD TECHNIQUE) (TABLE-3)

Source of variance	d.f.	Colony diameter		Per cent growth inhibition	
		SS	MSS	SS	MSS
Fungicide	7	120094.67	17156.381	182322.24	26046.035
Conc.	1	13.65	13.654	20.73	20.729
F^0 C	7	50.14	7.1634	76.13	10.8751
Error	64	0.209	0.003	0.318	0.0050

APPENDIX- VI

ANALYSIS OF VARIANCE FOR *IN VITRO* EFFICACY OF VARIOUS SYSTEMIC AND NON SYSTEMIC FUNGICIDES AGAINST *Fusarium Solani* AFTER 7 DAYS AT 28 ± 2^{0}C (POISONED FOOD TECHNIQUE) (TABLE-4)

Source of variance	d.f.	Colony diameter		Per cent growth inhibition	
		SS	MSS	SS	MSS
Fungicide	7	51397.23	7342.462	78337.50	11191.071
Conc.	1	751.90	75.896	1146.01	1146.008
F^0 C	7	1210.13	172.8758	1844.43	263.4901
Error	64	19.199	0.3000	29.263	0.4572

APPENDIX- VII

ANALYSIS OF VARIANCE FOR *IN VITRO* EVALUATION OF BOTANICALS FOR SOYBEAN ROOT ROT PATHOGEN *Rhizoctonia Solani* AT 28 $\pm$ 2^{0}C (POISONED FOOD TECHNIQUE) (TABLE- 5)

Source of variance	d.f.	Colony diameter		Per cent growth inhibition	
		SS	MSS	SS	MSS
Treatment	2	739.43	369.72	1100.23	550.12
Error	12	120.11	10.01	2.47	0.21

APPENDIX- VIII

ANALYSIS OF VARIANCE FOR *IN VITRO* EVALUATION OF BOTANICALS FOR SOYBEAN ROOT ROT PATHOGEN *Fusarium Solani* AT 28 $\pm$ 2^{0}C (POISONED FOOD TECHNIQUE) (TABLE-5)

Source of variance	d.f.	Colony diameter		Per cent growth inhibition	
		SS	MSS	SS	MSS
Treatment	2	1100.11	550.05	1726.30	863.15
Error	12	143.24	11.94	4.22	0.35

APPENDIX- IX

ANALYSIS OF VARIANCE FOR *IN VITRO* EVALUATION OF *Trichoderma viride* AND BACTERIA (*Bacillus* spp.) FOR ANTAGONISM TO SOYBEAN ROOT ROT PATHOGEN *Rhizoctonia solani* AT 28 $\pm$ 2^{0}C (DUAL CULTURE TECHNIQUE) (TABLE-6)

Source of variance	d.f.	Colony diameter		Per cent growth inhibition	
		SS	MSS	SS	MSS
Treatment	2	17377.25	8688.62	26815.65	13407.83
Error	12	1.62	0.13	2.04	0.17

APPENDIX- X

ANALYSIS OF VARIANCE FOR *IN VITRO* EVALUATION OF *Trichoderma viride* AND BACTERIA (*Bacillus* spp.) FOR ANTAGONISM TO SOYBEAN ROOT ROT PATHOGEN *Fhizoctonia solani* AT 28 $\pm$ 2^0C (DUAL CULTURE TECHNIQUE) (TABLE-7)

Source of variance	d.f.	Colony diameter		Per cent growth inhibition	
		SS	MSS	SS	MSS
Treatment	2	15581.66	7790.83	23678.82	11839.41
Error	12	0.59	0.05	1.01	0.08

APPENDIX- XI

ANALYSIS OF VARIANCE FOR EFFECT OF SEED TREATMENTS WITH BIOCONTROL AGENTS AND FUNGICIDES ON PER CENT GERMINATION, PER CENT ROOT ROT AND BIOMASS PRODUCTION IN SOYBEAN CULTIVAR `NRC-76` IN PATHOGEN INOCULATED SOIL (TABLE-8)

Source of variance	d.f.	Germination per cent		Per cent root-rot		Dry weight of plants (g)	
		SS	MSS	SS	MSS	SS	MSS
Treatment	11	3775.11	343.19	5067.45	460.68	62.07	5.64
Error	24	597.14	16.60	70.47	2.94	6.26	0.26

APPENDIX- XII

ANALYSIS OF VARIANCE FOR POPULATION DENSITY OF BIOCONTROL AGENTS c.f.u. x 10^5 / SOIL (TABLE-9)

Source of variance	d.f.	*Trichoderma viride*		Bacteria (*Bacillus* spp. Gram^{-ive})	
		SS	MSS	SS	MSS
Treatment	11	564.65	51.33	759.83	69.08
Error	24	1.65	0.07	2.04	0.08

APPENDIX- XIII

ANALYSIS OF VARIANCE FOR POPULATION DENSITY OF *R. solani* AND *F. solani* c.f.u. x 10^4 / g SOIL AND 10^5 / g SOIL RESPECTIVELY (TABLE-9)

Source of variance	d.f.	*Rhizoctonia solani*		*Fusarium solani*	
		SS	MSS	SS	MSS
Treatment	11	21.63	1.97	5.31	0.48
Error	24	0.69	0.03	1.83	0.08

APPENDIX- XIV

COMPOSITION OF MEDIA

1. **Modified PDA Triton X-100 medium (Budge and Whipps, 1991)**

Peeled potato	250.00g
Dextrose	20.00g
Agar-agar	20.00g
Distilled water	1000 ml
pH	7.00
(To add after autoclaving)	
Triton X-100	2 ml / litre
Chloramphenicol	60 ppm*
(*In place of chlorotetracycline)	

2. **0.1% Malt extract agar (MEA) medium :**

Used for *Trichoderma viride*

Malt extract	1.00g
Peptone	1.00g
Agar-agar	20.00g
Distilled water	1000 ml
(To add after autoclaving)	
Dicrystin	50 mg / litre
	Water

3. Potato dextrose agar (PDA) medium :

Peeled potato	250.00g
Dextrose	20.00g
Agar-agar	20.00g
Distilled water	1000 ml
pH	7.00
(To add after autoclaving)	
Dicrystin	100 ppM

4. Peptone PCNB medium (Nash and Snyer, 1962) :

Peptone	15.00g
KH_2PO_4	1.00g
$MgSO_4.7H_2O$	0.50g
Agar-agar	20.00g
Distilled water	1000 ml
pH	7.00
(To add after autoclaving)	
PCNB*	1000 ppm
Dicrystin	100 ppm

(* PCNB = Pentachloronitrobenzene)

5. King's 'B' agar medium (King's *et. al.,* 1962) :

Peptone	15.00g
KH_2PO_4	1.00g
$MgSO_4.7H_2O$	0.50g
Agar-agar	20.00g
Distilled water	1000 ml
pH	7.00
(To add after autoclaving)	
PCNB*	1000 ppm
Dicrystin	100 ppm