Bioformulations and Indigenous Plant Protection Measures in Enhancing the Vitalities of Bio-Control Agents for Induced Systemic Resistance Suppressing Asian Soybean Rust in India

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***Abstract*—** Thirteen different treatments were evaluated against soybean rust during 2009 and 2010 kharif season at Main Agricultural Research Station,Dharwad.The results revealed the significant superiority of seed treatment with recommended seed dressing dosage of Trichoderma harzianum @6g/kg with 106 cfu/g + Spray with Cow urine@10%+T.harzianum@0.5% recorded minimum (35.1) Per cent Disease Index (PDI) followed by 37.4, 38.9 PDI in case of spray with Cow urine @10% + potassium phosphonate@0.3% and neem oil@1% respectively. However, minimum PDI was recorded in Hexaconazole @ 1ml/l (30.5) which are statistically on par with each other. The maximum incidence of 87.8 PDI was recorded in control. The maximum seed yield of 18.06q/ha was recorded in Hexaconazole@0.1% followed by Seed Treatment with Trichoderma harzianum @6g/kg+ Spray with Cow urine@10%+T.harzianum@0.5% (17.15q/ha) which are statistically on par with each other. The minimum seed yield was recorded in untreated check (9.06q/ha). Among the bio-intensive strategies, reducing sugars was maximum(0.737%) in seed treatment with Trichoderma harzianum @6g/kg+ Spray with Cow urine@10%+T.harzianum@0.5% followed by 0.707% in case of seed treatment with Pseudomonas fluorescens @10g/kg with 108 cells/ml + Spray with Cow urine@10%+ Pusedomonas fluorescens @0.5%.The minimum reducing sugars was recorded in untreated control (0.071%). With respect to non reducing sugars, seed treatment with Pseudomonas fluorescens @10g/kg+ Spray with Cow urine@10%+ Pusedomonas fluorescens @0.5% recorded maximum (10.53%) followed by 9.59% in case of Trichoderma harzianum @6g/kg+ Spray with Cow urine@10%+T.harzianum@0.5%. The minimum non reducing sugars percent was recorded in untreated control (1.22%). The peroxidase activity ranged between 25 to 70 Kda. The maximum peroxidase activity was recorded in cow urine @ 10% and neem oil@1%. We also studied the expression of polyphenol oxidase which is the key factor for upregulation of defense genes in Induced Systemic Resistance (ISR) against rust pathogen. The maximum polyphenol activity was recorded in Cow urine@10% +neem oil @ 5% followed by cow urine @10% +Trichoderma viride@0.5% and neem oil@1%.The polyphenol activity ranged between 55 to 110Kda.There was no expression of catalase activity in all the treatments.

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I. Introduction

THE Asian soybean rust (*Phakopsora pachyrhizi* Syd*)* is the economically important disease not only in the Sub continent but also rest of the soybean growing regions of the world. The predominantly associated pathogen *Phakopsora pachyrhizi* has been known to drastically reduce yields in Asia. In areas where the pathogen occurs in most virulent form yield losses up to 80% have been reported (1). Basically the pathogen was confined to eastern hemisphere before it had appeared in epiphytotic form in Hawaii region in 1994. At present the pathogen has been reported from different continents such as Africa, Asia, Australia, South America and Hawaii. The rapid spread of *P. pachyrhizi* and potential for severe yield losses makes this, the most destructive foliar disease of soybean. Soybean rust could have a major impact on both total soybean production and production costs in the India. In India, the disease was first reported on soybean in 1951 (2).Two *Phakopsora* species are known to cause soybean rust (3).The more aggressive species is *P. pachyrhizi*, known as the Asian soybean rust. *P. meibomiae*, the less virulent species, has only been found in limited areas in the Western hemisphere, and it is not known to cause severe yield losses in soybean.

Most research on control has been focused on the use of fungicides and host plant resistance. Some cultural practices have been recommended that minimize the impact of rust (4) and (5). The recommendations differed, but were based upon avoiding the conditions that promote disease development or were practices that optimized overall yields. Research on biological control has been limited in the management of soybean rust. In recent years the studies on use of Indigenous Technology Knowledge (ITK) measures in the managing of the crop diseases have been demonstrated successfully in crops like sorghum, tomato, banana and black pepper (6), (7) and 8. The soybean growers of the subcontinent are seriously facing the severe infestation of rust disease in the last few years with

a yield loss ranging from 30-100%. Lack of resistant cultivars at present for Asian soybean rust and continuous application of fungicides has further aggravated the concern over pesticide resistance.Keeping these points in view the investigation comprised of screening the Indigenous Technology Knowledge (ITK) measures against Asian soybean rust in India with an aim of enhancing the productivity in terms of both quality and quantity of soybean in India.

II. Material And Methods

The present study comprised of thirteen different bioformulations taken up at Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad during *Kharif* 2009 and 2010. The treatments were applied thrice with first one immediately after the appearance of rust symptoms in the field and subsequently formulations were applied at 10 days interval. Thirteen different treatments comprising of botanicals, bioagents and micronutrients were evaluated against soybean rust. The botanicals and cow urine were applied thrice starting from disease appearance and subsequently at 10 days interval. The different plant based extracts were prepared by dipping the crushed leaves in cold water for 30min and later filtered through muslin cloth.

The details of the experimentation comprised of 13 treatments with most susceptible cultivar JS 335 replicated three times with a plot size of 2.7 x 4.0 sq.mt. The sowing was done on 6-7-2009 & 26-7-2010.The different treatments were applied after the appearance of first symptom i.e on 04-09-2009, and later on 18-9-2009, 25-9-2009, 2-10-2009 during 2009 and 26-09-2010,6-9-2010 and 14-10-2010 during 2010.The seasonal conditions revealed late on set of monsoon in the region. The total rainfall received was 926.8mm spread over 60rainy days in 2008. In 2009, total rainfall received was 1022.6mm spread over 66 rainy days. The receipt of above normal rainfall during August and September months coupled with high relative humidity and intermittent rainfall resulted in epiphytotics of rust during the season. Hence, the season was more congenial for development of strategies for rust management. The rust infected leaves were collected and harvested the uredospores by macerating the leaves in water and such inoculum is sprayed in the evening hours. For enzyme studies, seedlings of soybean variety JS 335 were raised in pot culture studies in glass house.The plants were inoculated with different treatments by leaf infiltration method (9) and later after one week after infiltration the plants were challenge inoculated with rust spores by stapling the infected leaves at 4-5 leaf stage (10).Observations on rust severity were recorded as per the scale of (11). when the crop was 85 days old. For scoring the intensity of rust, ten plants were randomly selected in the central rows of the plots. The percent disease index (PDI) was further calculated using the formula of Wheeler (12) and seed yield was also recorded at harvest (q/ha). The economic analysis of treatments was carried out taking into cognize of two year yield data on pooled basis and benefit cost ratio has been worked for all the treatments to find out the economic feasibility of treatments. The data were statistically analyzed using RBD design (13).

III. Results And Discussion

A. Development Bio-Intensive Management of Soybean Rust

The present study comprised of thirteen different treatments involving botanicals, bioagents and micronutrients. During 2009, among the bio-intensive strategies, seed treatment with *Trichoderma harzianum* @6g/kg+ Spray with Cow urine @ 10% + *T .harzianum* @ 0.5% recorded minimum (37.9) Per cent Disease Index(PDI) followed by 39.6,40.6 PDI in case of spray with Cow urine @10% + potassium phosphonate@0.3% and neem oil@1% respectively. However, minimum PDI was recorded in Hexaconazole @ 1ml/l (32.2) which differed significantly from rest of the treatments. The maximum incidence of 86.7 PDI was recorded in untreated check. The maximum seed yield of 17.33q/ha was recorded in Hexaconazole@0.1% followed by seed treatment with *Trichoderma harzianum* @6g/kg+ Spray with Cow urine@10%+*T.harzianum*@0.5% (16.82q/ha) which are statistically on par with each other. The minimum seed yield was recorded in untreated check (8.20q/ha).

During 2010, among the bio-intensive strategies seed treatment with *Trichoderma harzianum* @6g/kg+ Spray with Cow urine@10%+*T.harzianum*@0.5% recorded minimum (32.2) Per cent Disease Index (PDI) followed by 35.1, 37.1 PDI in case of spray with Cow urine @10% + potassium phosphonate@0.3% and neem oil@1% respectively. However, minimum PDI was recorded in Hexaconazole @ 1ml/l (28.8) which are statistically on par with each other. The maximum incidence of 88.9 PDI was recorded in untreated check. The maximum seed yield of 18.78q/ha was recorded in Hexaconazole@0.1% followed by ST with *Trichoderma harzianum* @6g/kg+ Spray with Cow urine@10%+*T.harzianum*@0.5% (17.48q/ha) which are statistically on par with each other. The minimum seed yield was recorded in untreated check (9.93q/ha).

Among the bio-intensive strategies, the pooled analysis over two years also revealed the significant superiority of seed treatment with *Trichoderma harzianum* @6g/kg+ Spray with Cow urine@10%+*T.harzianum*@0.5% recorded minimum (35.1) Per cent Disease Index (PDI) followed by 37.4, 38.9 PDI in case of spray with Cow urine @10% + potassium phosphonate@0.3% and neem oil@1% respectively. However, minimum PDI was recorded in Hexaconazole @ 1ml/l (30.5) which are statistically on par with each other. The maximum incidence of 87.8 PDI was recorded in untreated check. The maximum seed yield of 18.06q/ha was recorded in Hexaconazole@0.1% followed by ST with *Trichoderma harzianum* @6g/kg+ Spray with Cow urine@10%+*T.harzianum*@0.5% (17.15q/ha) which are statistically on par with each other. The minimum seed yield was recorded in untreated check (9.06q/ha).

The economic analysis of the project revealed that among the bio-intensive strategies seed treatment with *Trichoderma harzianum* @6g/kg+ Spray with Cow urine@10%+*T.harzianum*@0.5% recorded maximum net income of Rs.31, 850/ha with benefit cost ratio of 1:2.62 followed by spraying with cow urine @10% (Rs.24, 650 net

income & 1:2.24).The minimum net income of Rs.12150 with C:B ratio of 1:1.16 was recorded in untreated control.

B. Influence of different bio-intensive treatments on biochemical parameters

We also studied the role of biochemical parameters in triggering defense genes and increasing in seed yield apart from bringing down the disease pressure. Among the bio-intensive strategies, reducing sugars was maximum(0.737%) in seed treatment with *Trichoderma harzianum* @6g/kg+ Spray with Cow urine@10%+*T.harzianum*@0.5% followed by 0.707% in case of seed treatment with *Pseudomonas fluorescens* @10g/kg+ Spray with Cow urine@10%+ *Pseudomonas fluorescens* @0.5%.The minimum reducing sugars was recorded in untreated control (0.071%).

With respect to non reducing sugars, seed treatment with *Pseudomonas fluorescens* @ 10g/kg + Spray with Cow urine@10% + *Pusedomonas fluorescens* @0.5% recorded maximum % non reducing sugars (10.53) followed by 9.59% in case of *Trichoderma harzianum* @ 6g/kg+ Spray with Cow urine@10%+*T.harzianum* @ 0.5%.The minimum non reducing sugars % was recorded in untreated control (1.22%).

C. Role of defense genes in biochemical basis of resistance against rust by ITK methods.

The role of different enzymes in triggering the host defense was studied by the use of Indigeneous Technology Knowledge measures by employing Lowry's Method and Poly Acrylamide Gel Electrophoresis(PAGE) for assessing the total protein and estimation of peroxidase, polyphenol oxidase and catalase activity. The peroxidase activity ranged between 25 to 70Kda (fig. 1). The peroxidase activity was better in all the bio-intensive treatments indicating triggering of host defense genes. The maximum peroxidase activity was recorded in cowurine@10% and neem oil@1%(Fig 1). We also studied the expression of polyphenol oxidase which is the key factor up regulation of defense genes in Induced Systemic Resistance(ISR) against rust pathogen. The maximum polyphenol oxidase activity (fig. 2) was recorded in Cow urine@10% +neem oil @5%, Cow urine@10% +*Trichoderma viride*@0.5% and neem. There was no expression of catalase activity (fig. 3) in any names of treatments. This signifies the absence of catalse pathway in ISR against soybean rust. Based on the expression of type of defense genes involved the studies brought for the first time a new information on salicylic acid based pathway in inducing resistance in the soybean using ITK measures. This information will be a key factor in developing ISR elicitors against soybean rust. The use of ITK measures and bioformulations enhanced the plant growth promotion resulting in higher seed yield. Thus, it will further help to reduce pesticide application by the farmers for managing soybean rust and minimizes the cost of production.

In the present investigation use of chemical elicitors like Multi K and MnSo4, plant based extracts like *Adathoda vasica*, *Prosopis juliflora*,*Pongamia pinnata* oil, Neem oil, Cristol and bioagent *Trichoderma harzianum* in combination with cow urine triggered the host defense resulting in significant reduction in disease pressure when compared to untreated check (Table 1).The application of neem oil alone has not resulted in significant reduction in disease pressure. Thus, the study clearly indicated benefits of combined application of cow urine along with along with bioformulations with a better protection against Asian soybean rust. The application of cow urine along with biorationals recorded at par seed yield with chemical control. This is a very good indication to develop an eco-friendly bio-intensive disease management strategy against Asian soybean rust in India. There is a need to further explore the role of defense genes being triggered by use of these elicitors leading to Induced Systemic Resistance (ISR) in soybean against Asian rust. The present investigations are the first line of research in managing Asian soybean rust by utilization of ITK measures. The successful management of panama disease of banana caused by *Fusarium oxysporum* f. sp. c*ubense*, foot rot of black pepper, TMV of tobacco and Fusarium wilt of tomato by use of neem based products and ITK measures has been reported by (16),(17) and (18), also reported the efficacy of Mahapanchaya Gavya in controlling damping off in tomato. The chemical elicitors like MnS04, Muti-k or plant based extracts like *A.vasica*. *Pongamia pinnata* oil and bioagent like *Trichoderma harzianum* along with cow urine be used in developing Integrated Disease Management strategies against Asian Soybean rust in India which will help in reducing the chemical pesticides in long term sustainable management. The present findings drawn the first line of research on utilization of Indigenous Technology Knowledge in managing rust and enhancing both yield and quality parameters of soybean in India.

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TABLE I
BIO-INTENSIVE MANAGEMENT OF SOYBEAN RUST CAUSED BY *PHAKOPSORA PACHYRHIZI(2009-10)*

Treatments	Observations		
	PDI	Seed yield (q/ha)	C:B ratio
Spray with Cow urine@10% + *Adathoda vasica* @5%	44.1 (41.6)	11.82	1:1.20
Spray with Cow urine@10% + *Prosopis juliflora* @5%	46.3 (42.9)	12.65	1:1.66
Spray with Cow urine@10% + neem oil @0.5%	44.1 (41.6)	12.55	1:1.85
ST with *Trichoderma harzianum* @ 6g/kg + spray with Cow urine @10% + *Trichoderma harzianum*@0.5%	35.1 (36.3)	17.15	1:2.62
ST with *Pseudomonas fluorescens* @10g/kg+ Spray with Cow urine @ 10% + *P. fluorescens* @0.5%	42.9 (40.9)	13.38	1:2.03
Spray with Cow urine @ 10% + *Trichoderma harzianum*@0.5%	42.7 (40.8)	13.21	1:1.94
Spray with Cow urine@10% + Potassium Phosphonate @0.3%	37.4 (37.6)	13.74	1:1.79
Spray with Cow urine@10% + Cristol 56SL @0.5%	41.3 (39.9)	14.37	1:1.90
Cow urine @ 10% + *Pongamia pinnata* oil @ 0.5%	39.5 (38.9)	13.54	1:1.65
Cow urine @ 10%	42.8 (40.9)	14.26	1:2.24
Neem oil @ 1%	38.9 (38.6)	14.18	1:2.07
Hexaconazole @ 0.1%	30.5 (33.5)	18.06	1:2.94
Control	87.8 (69.6)	9.06	1:1.16
S. EM±	1.34	53.53	
CD (5%)	4.13	133.8	
CV (%)	17.32	19.40	

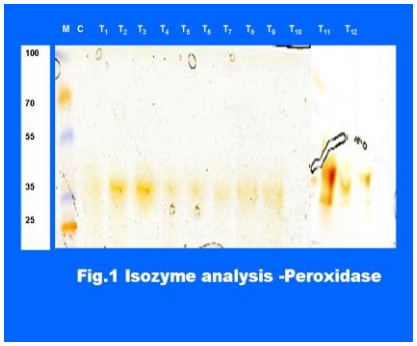

Fig.1 Isozyme analysis -Peroxidase

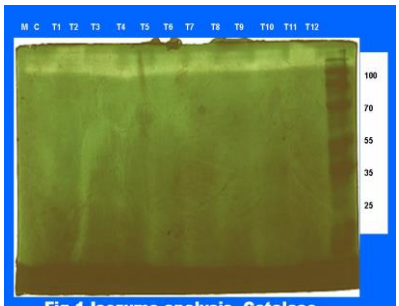

Fig.1 Isozyme analysis -Catalase

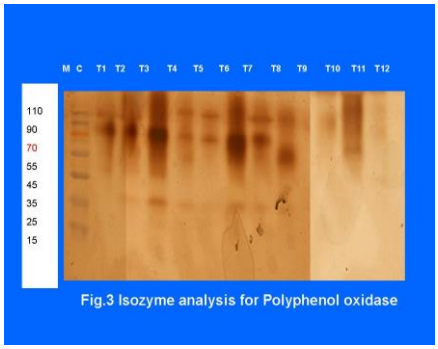

Fig.3 Isozyme analysis for Polyphenol oxidase

M- Marker, C- Control, T1-Cow urine@10% + Adathoda vasica @5% T2-Cow urine@10% + Prosopis juliflora @5%.T3-Cow urine@10% + neem oil @0.5% T4-Trichoderma harzianum @ 6g/kg + spray with Cow urine @10% + Trichoderma harzianum@0.5%.T5-Pseudomonas fluorescens @10g/kg+ Spray with Cow urine @ 10% + P. fluorescens @0.5%. T6-Cow urine @ 10% + Trichoderma harzianum@0.5% T7- Cow urine@10% + Potassium Phosphonate @0.3% T8-Cow urine@10% + Cristol 56SL @0.5% T9- Cow urine @ 10% + Pongamia pinnata oil @ 0.5% T10-Cow urine @ 10% T11-Neem oil @ 1% T12-Hexaconazole @ 0.1%.