Biocontrol of Damping off in Withania Somnifera (L) Dunal

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Abstract

Present work was aimed to find the effect of biocontrol agents on the Damping off in Withania Somnifera which becomes victim of many soil borne fungi. Damping off a major disease at seedling stage to curb various conventional pesticides was used which carried negative impact. During study seeds were treated first with Azotobacter that showed moderate germination followed by combined treatment of Azotobacter and trichoderma which showed very high. Also it was found that disease frequency was high in replicas of control because no biocontrol / biofertilizer were applied. Trichoderma alone had moderate effect on Damping off. Finally, Azotobacter and trichoderma synergistically showed best results against the disease as former helped in growth parameters while later fights against causative pathogen.

Keywords: Withania somnifera, pesticides, bio fertilizer. Trichomoderma.

INTRODUCTION

Withania somnifera, known commonly as Ashwagandha, Indian ginseng, poison gooseberry or winter cherry, is a plant in the Solanaceae or nightshade family. Several other species in the genus Withania are morphologically similar. It is used as an herb in Ayurvedic medicine. Withania somnifera is prone to several pests and diseases. Leaf spot disease caused by Alternaria alternata is the most prevalent disease, which is most severe in the plains of Punjab, Haryana, and Himachal Pradesh. Biodeterioration of its pharmaceutically active components during leaf spot disease has been reported. The Choanephora cucurbitarum causes a stem and leaf rot of Withania somnifera Atreehopper, feeds on the apical portions of the stem, making them rough and woody in appearance and brown in colour. The apical leaves are shed and the plant gradually dies. The carmine red spider mite (Tetranychus urticae) is the most prevalent pest of the plant in India.

Review of literature

Withania somnifera locally known as Ashwangandha which means sweat of Horse, is an important medicinal plant in traditional Ayurvedic system of medicines (Devi, p.u – 1996), its root extract is widely used as a tonic and in numerous ailments (chopra *et al.,* 1956). It is cosmopolitan and grows throughout drier parts and of subtropical India considered a potential crop (Nigham K.B. 1989). As per pathological studies the plant becomes victim of various contact and systematic diseases. The pathogen may be bacteria, mites, Aphides and lady bird beetle (Bruhd. G.C 1989) Several root borne fungi are known to be plant pathogens which cause root and seedling disease on crops (Marten, *et al,.* 1988) Janadhan K.K. 2002).

Damping off, single term used to describe under ground, soil line or crown rots of seedlings due to unknown causes (Guptha *et al.,* 1993). In order to curb

this dreadful disease, various conventional pesticides like Dithane m-45, cuman – L, were tried (Guptha *et al.,* 1993). Their usage carries more adverse impacts on environment (Mastsumra, F, 1977As an alternate Strategy which is an EPA based Biopesticide programme has to be adopted (Greg. J.B, Boland – 1998).

MATERIAL AND METHODS

Media Preparation:
Media requirements:
Nutrients
Water
Source of energy
Source of C, N, S, P.
Minerals (Ca^{2+}, Mg^{2+}, Na^{+})
Vitamins and growth factors.
B. **Equipments:**
→ **1000ml Erlenmeyer Flask**
Balance weighing
Autoclave
Sterile 100 mm pettri dishs.
37ºC incubator
55º C water bath media.
Steps in media preparation:
Dry media was weightedout and dissolved .It was then dried and autoclaved and finally poured into pouring plates
General Bacterial Media:
Nutrient Broth: An empirical medium of general use for cultivation of most bacteria.
Components of Medium:
Peptone – 10g, Meat Extracted lab lemco -10g, Nacl- 5g, distilled H_2O – 1000ml
Procedure: Weigh ingredients and heat in steamer until dissolved.
When cool adjust PH. 7.5
Auto clave at 121ºC
Filtration
Adjust PH with NaCl or Hcl to 7.2.
Nutrient Agar.
Nutrient broth (p^H 7.2) – 1000ml
Agar – 15 gm
General Fungal Media: (PDA) Potato Dextrose Agar is one of the best media for growth of fungi
Azotobacter Media: Azotobacter grows on surface pellicle on shallow layers of fluid medium of the following composition (%w/v)
Glucose – 1
K_2HPO_4 – 0.1
$MgSO_4, 7H_2O$ = 0.02
$CaCo_3$ – 0.1
Nacl – 0.02
$Na_2M0_0O_4$, $2H_2O$ – 0.0005
Procedure: Components were dissolved in distilled water and the medium was sterilized by Autoclaving at 121ºc for 10 min. Medium, was solidified by the addition of 2 % Agar.

Experimental layout: There were four treatments with three replicas each

S. No	Treatment	Replica	Biocontrol agent
1.	**Control (c)**		-
	T_1	C_1	-
	T_2	C_2	-
	T_3	C_3	-
2.	Azotobacter (AZ)		Azotobacter
	T_4	Az_1	-
	T_5	Az_2	-
	T_6	Az_3	-
3.	Trichoderma (TD)		
	T_7	TD_1	-
	T_8	TD_2	-
	T_9	TD_3	

RESULTS

The present work aimed ay finding the effect of biocontrol agents on Damping off in Withania somnifera. The whole experiment set up was divided into four treatment sets, which are shown in the Tables and Figures below.

The basic strategy of seed treatment is to establish a large population of Antagonastic on seed as a protactant. The germination percentage in the control is less because of low nutrient content in the soil and high risk of pathogen attack, because no bio agent was applied, however seeds treated with Azotobacter. Shows moderate germination, as Azotobacter is considered as the best biofertiliser. While in Azotobacter and Trichoderma terated seed the germination is very high because of application of both, bio-fertiliser and bio control agents. After planting the seeds, the antagonastic colonise the seed coat and protect the young germinating seedling against the attack of fungal pathogen by competition. Wilson, C.L. and Pusy, P.L (1985).Potential for biological control of post harvest plant diseases, Plant Diseases., 69:375-378.

DISCUSSION

Present work aimed at finding the effect of Biocontrol agents (Azotobacter and Trichoderma) on percentage of inoculants effects by Damping off in withania somnifera .Experiment was carried out into four treatment sets: Control (without innoculants), Azotobcter treatment, trichoderma treatment and Azotobacter -trichoderma treatment.

Seeds have shown a significant increase in the germination percentage from untreated seeds (control) to those treated with Azotobacter and trichoderma both singly and mixed with seeds as is suggested also by (Transom. A 1989). Also the nutrient content in natural soil is less and a high risk of pathogen attack because protectant was applied (pantwardhan. S. 1999). Moreover

Table 1. Germination percentage of witiiania somnifera (l) dunal
Observation 1

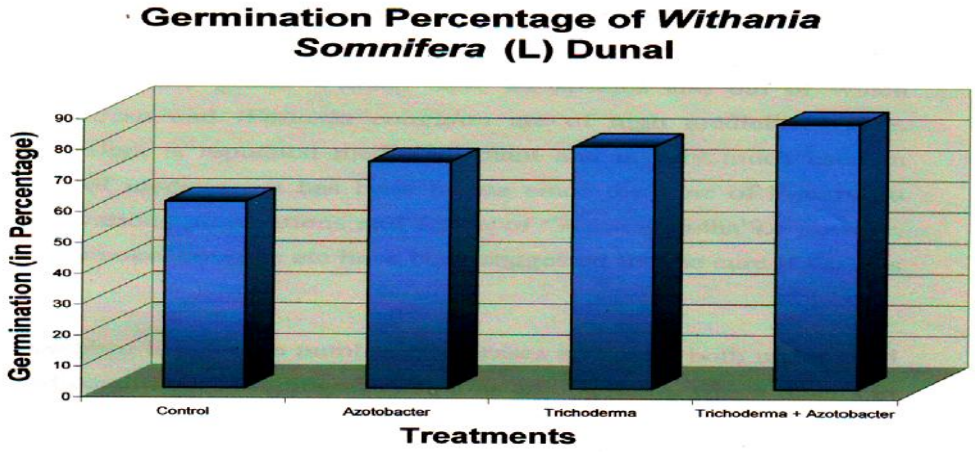
S. No	No.of seeds sown	No. of seeds germinate	Mean	Germination Percentage	Standard devotion	Standard error
1	Control (C) C1 C2 C3	100 100 100	63 57 61	60.33	3.05	1.52
2	Azotobaerter(A Z) AZ1 AZ 2 AZ3	100 100 100	74 69 77	73.33	4.04	2.02
3	Trichodeima(TD) TD1 TD2 TD3	100 100 100	82 75 78	78.33	3.51	1.75
4	Azotobacter+Tri choderma (AZ+TD) (AZ+TD) 1 (AZ+TD) 2 (AZ+TD) 3	100 100 100	85 89 83	85.6	3.05	1.52

Table 2. Survival percentage of withania somnifera (L) Dunal
Observation 2

S. No	No. of seeds sown	Total No. of seedling	Mean	Total No. live seedling	Mean	Survival percentage	Standard Deviation	Standard error
1	Control (C) C1 C2 C3	63 57 61	60.33	49 47 46	47.33	78.56	1.52	0.76
2	Azotobaerter(AZ) AZ1 AZ 2 AZ3	74 69 77	73.33	68 60 69	66.66	89.54	4.93	2.46
3	Trichodeima(TD) TD1 TD2 TD3	82 75 78	78.33	78 72 75	75	95.7	3	1.50
4	Azotobacter+Tri choderma (AZ+TD) (AZ+TD) 1 (AZ+TD) 2 (AZ+TD) 3	85 89 83	85.6	83 87 81	83.66	97.74	3.05	1.52

Table 3. Damping off percentage of withania somnifer.4 (L) Dunal
Observation 3

S. No	No.of seeds sown	Total No. of seedling	Mean	Total No. dead seedling	Mean	Damping of percentage	Standard Deviation	Standard error
1	Control (C) C1 C2 C3	63 57 61	60.33	14 10 15	13	21.54	2.64	1.32
2	Azotobaerter(AZ) AZ1 AZ 2 AZ3	74 69 77	73.33	6 9 8	7.6	10.46	1.52	0.76
3	Trichodeima(TD) TD1 TD2 TD3	82 75 78	78.33	4 3 3	3.33	4.25	0.57	0.28
4	Azotobacter+Tri choderma (AZ+TD) (AZ+TD) 1 (AZ+TD) 2 (AZ+TD) 3	85 89 83	85.6	2 2 2	2	3.05	00	00



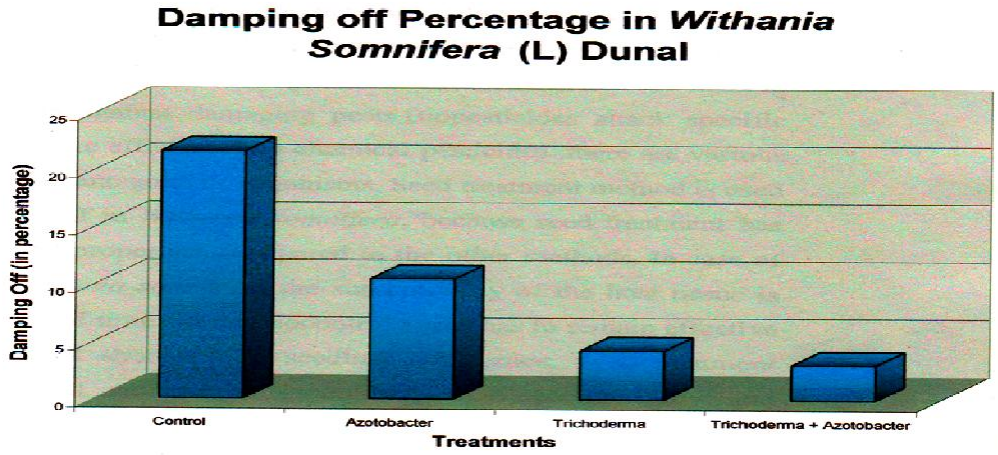


Plate: Isolation of Pathogen

Plate: Culturing of Rhizoctonia Solani

seedlings that reach to reproductive stage in correspondence to the number of seeds sown showed a marked difference. Biopesticides applied to seeds acted as Protectants saving under surface part from pathogen (phythium sp.). However sometimes inorganic pesticides-to enhance the germination may be inevitable and integrated with but only after few days of Biopesticide treatment (Sankoram J. 1988). The basic strategy of seed treatment is to establish a large population of Antagonastic on seed as a protectant (Baker. K.F. 1987).

Azotobacter is considered as best biofertilizer when applied secretes some minute quantities of enzymes, vitamins, growth regulators. Fungicides besides fixing nitrogen for plant (Fravel D.R. 1988). Present plant is much prone to number of diseases and pests both under field and cultivated conditions. Damping off is serious among them which results in heavy loss of plant population. Causative agents (phythium sp., Rhizoctonia solani) of disease can be curtailed by various chemical pesticides - protecting environmental balance hence biopesticides are viewed as an alternative. Seed treatment method is used against the damping off in withania somnifera because treated seed has unique advantages and properties compared to other methods. In case of Damping Off in withania somnifera the susceptibility of the host tissue is limited to short period of time (Weller D.M. 1988) so biocontrol agent has to remain effective only from sowing until shortly after seedling emergence. The result inferred disease frequency is more in replicas of control in absence of biocontrol /biofertilizer while in Azotobacter treatment germination and survival percentage is moderate but damping off was also recorded there. Trichoderma however, is so efficient and fruitful against the Damping off. Finally Azotobacter and trichoderma synergistically shows significant and best result against the disease as Azotobacter helps in growth parameters while trichoderma fights against pathogen as is also noted by Howell. L.R. 2002

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