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Protocol for Extraction of Silkworm (*Bombyx mori* L.) Pupal Residue Bio Soft Descent (SPRBD) and its Properties

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Abstract

The present study was undertaken with an objective to utilize silkworm pupae disposed by silk reeling units as a waste. Designed special equipment was used for extraction of silkworm pupal residue bio soft descent (SPRBD) from silkworm pupal residue collected from different reeling of Karnataka State. The extraction of SPRBD was carried out by applying different pressures of 16, 18 and 20 tonnes on silkworm pupa. Significantly higher quantity of 2.61 Kg (52 %) silkworm pupal residue biosoft descent was extracted at 18 tonnes pressure. The next best treatment yielded 2.20 Kg (44 %) of SPRBD at 20 tonnes pressure. The SPRBD appeared creamish in colour with badam drink odour and slightly turbid in nature. The microbial load of SPRBD at 10^{-1} dilution was highest for bacteria (40 CFU/ml) followed by actinomycetes (5 CFU/ml) and yeast (3 CFU/ml). However, no fungus was found in SPRBD. The biochemical composition of SPRBD *viz.,* moisture percentage (8.6 %), total protein (60.77 %), carbohydrate (5.63 %), total fat (9.20 %), ash (2.27 %), glucose (1.25 %) and vitamin E (1.56 mg). The chemical composition of SPRBD *viz.,* pH (6.8), electrical conductivity (1.36 dsm^{-1}), organic carbon (18 %), nitrogen (N) (3.41 %), phosphorous (P) (0.95 %), potassium (K) (0.59 %), calcium, magnesium and sulphur were 0.32 , 0.29 and 0.17 per cent. Sodium (Na), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and boran (B) were 0.07 per cent, 119.33 ppm, 2.97 ppm, 176 ppm, 5.13 ppm and 1.36 ppm, respectively. *In vitro* evaluation of SPRBD against different fungal pathogens *viz., Alternaria solani*, *Fusarium oxysporium, Pythium* spp., *Rhizoctania soloni* and *Sclerotium rolfsii* recorded more than 50 per cent mycelial inhibition of all the test pathogens at 20 per cent concentration of SPRBD. Under glass house conditions lowest per cent disease severity was observed in the tomato plants treated with 10 per cent SPRBD.

Keywords : Silkworm, Pupal residue, SPRBD, Biosoft descent

The silkworm, *Bombyx mori* L., an economically important insect, is reared for getting huge quantity of silk. Pupa, the third developmental stage of the life cycle of silkworm, accounts for major portion of the cocoon weight and inevitable byproduct generated in large quantities (75-85%) in silk reeling industries. It is estimated that, 1.5 lakh tonnes of silkworm pupae are produced annually in case of mulberry silkworm in India (CSB, 2017).

In order to ensure a profitable sericulture activity, it is necessary to process the secondary and waste products of sericulture industry to obtain biologically active substances which in turn used in pharmaceutical, cosmetic, paper and cellulose and food industries (Velayudhan *et al.,* 2008).

The by-products presently felt as wastes, can be put to better use in generating the value based products and thereby catapult the industry to a more profitable and economically viable spot. Through integrated approach, the pupa can be converted into marketable product which makes sericulture more profitable, can reduce production cost, pollution, recycle resources to cater the ever growing population and their demanding wants (Manohar Reddy, 2008).

The idea of by-product utility can be highly useful to sericulture industry and help in not only increasing the socio economic status of the rearers but also effective conversion of wastes / by-products into highly useful biological products which in turn will proportionately reduce the silk production cost and environmental

problems. The major difficulty in the utilization of spent silkworm pupae is that, it cannot be stored for long periods because of emission of bad odour. This is because of the presence of more than 70 per cent moisture in pupa. As such drying requires lot of time and energy due to presence of high amount of moisture in the pupa. Hence, the present study was undertaken by collecting silkworm pupae from different reeling units of Karnataka and development of protocol for extraction of silkworm pupal residue biosoft descent (SPRBD) from silkworm pupal residue without using electric power.

Methodology

a. Selection of pupae

Silkworm pupae were collected from reeling units situated in Vijyapura, Sidlaghatta and Chintamani of Chikkaballapur district, Karnataka state. Collected pupae were placed in the plastic containers fitted with lids. In the laboratory, healthy pupae were sorted and selected for extraction.

b. Extraction of silkworm pupal bio soft descent

A moisture removal device fitted with hand operated hydraulic system and perforated drum, developed in consultation with the Department of Mechanical Engineering, Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru was used in extraction.

The pupa was placed in perforated metal drum, and covered by plastic bag. Before filling the drum, the containers bulk density in which the pupa was filled was calculated (Bulk density was calculated in order to know the amount of pupae taken in a container). Later, the drum was placed under the pressures of 16, 18 and 20 tonnes to press the pupae in order to extract the pupal bio soft descent. During pressing, the pupal bio soft descent was collected in the plastic bag placed around the perforated metallic drum. Followed by this, the extract was transferred to a container and the weight of the extracted pupal bio soft descent was recorded. The pupal residue present in the drum after pressing was taken out and weight of the pupal residue was noted. Then the extract was preserved in refrigerator at 5^0C and further utilized for analysis. The silkworm pupal residue bio soft descent and silkworm pupal residue was used for value addition.

c. Physical properties and microbial load of silkworm pupal residue bio soft descent (SPRBD)

The physical properties *viz.,* colour and turbidity was analyzed by following spectophotometric and nephlometric method respectively.

The isolation and enumeration of different microorganisms from silkworm pupal residue bio soft descent was done by using serial dilution and standard plate count technique. Ten ml of freshly collected and air dried silkworm pupal residue bio soft descent sample was transferred to 90 ml sterile water blank and mixed well. 20 ml of media *viz.,* Nutrient agar and Potato dextrose agar bacteria and fungi, Martin rose bengal agar for yeast and acitinomycetes was poured into plates. The plates were rotated twice in clockwise and anticlockwise direction for uniform distribution of the inoculums. After solidifications of the media, plates were kept for incubation in an invert position at 30 ± 1^0C for 2-4 days and emerged colonies were counted.

d. Biochemical analysis

Silkworm pupal residue bio soft descent was subjected to biochemical analysis by using standard protocols mentioned below.

Estimation of moisture (AOAC, 2007) : Moisture was determined by taking 10 ml of sample in petridish and dried in an oven at 60 ^{0}C till the weight of petridish with its content was constant. Each time before weighing, the petridish was cooled in desiccator.

Estimation of protein (AOAC, 2007) : The protein content of the sample was estimated as per cent total nitrogen by the Micro-kjeldahl procedure. Protein per cent was calculated by multiplying the per cent nitrogen by the factor 6.25.

Estimation of fat (AOAC, 2007) : Fat was estimated as crude ether extract using moisture free samples. The solvent was removed by evaporation and the residue of fat was weighed. The soxtherm equipment was used.

Estimation of total ash (AOAC, 2007) : The ash content of sample was obtained by dry ashing the samples completely by heating it over a flame.

Computation of carbohydrate (AOAC, 2007) : Carbohydrate content was calculated by differential method.

Glucose (%) : Glucose was estimated in acidic as well as in alcoholic extract by GOD-POD (Glucose oxidase-Peroxidase) methods.

Vitamin E : HPLC (high-performance liquid chromatography) is generally the method for the analysis of Vitamin E (Desai and Machlin, 1985).

e. Chemical analysis

Silkworm pupal residue bio soft descent was subjected to chemical analysis by using standard protocols mentioned below.

pH : pH was determined in 1: 10 organic material (powdered) water suspension by using a glass electrode pH meter by potentiometric method (Jackson, 1973).

Electrical Conductivity (EC) : Electrical conductivity was determined from the filtrate 1:10 organic material water suspension using a conductivity bridge (Jackson, 1973).

Organic carbon (OC) : Organic carbon content was determined by using Walkley and Black's (1934) method.

Total nitrogen : Total nitrogen was estimated by using the microkjeldahl method as outlined by Piper (1966) using Automatic Kjeldahl set.

Phosphorus and other nutrient elements : Nutrients *viz.,* P, K, Ca, Mg and micro nutrients *viz.,* Zn, Cu, Fe, Mn were determined in the sample obtained by diacid digestion method (Piper, 1966).

Total phosphorus : Total phosphorus was determined using Vanadomolybdic yellow colour method (Jackson, 1973).

Total potassium : Total potassium was determined using flame photometer method (Jackson, 1973).

Total Calcium and Magnesium : Total Ca and Mg were determined using Versenate titration method (Jackson, 1973).

Total Sulphur : Sulphur content in the di-acid digested sample was estimated by turbidometric method as outlined by Jackson (1973).

Micronutrients : The contents of iron, manganese, zinc and copper was determined by using atomic absorption spectrophotometer with appropriate hallow cathode lamps (Linday and Norwell, 1978).

f. *In vitro* evaluation of silkworm pupal residue bio soft descent (SPRBD)

The present investigation was aimed to study the anti-fungal activity of silkworm pupal bio soft descent. Silkworm pupal bio soft descent was used in different concentrations and in triplicates against different fungal pathogens.

***In vitro* assay for anti-fungal activity**

Antifungal activity of silkworm pupal residue bio soft descent (SPRBD) was determined by disc diffusion assay. 1 ml of various concentrations of silkworm pupal residue bio soft descent *viz*., 5, 10, 20, 30, 40 and 50 per cent concentration was added to the petri plates containing potato dextrose agar (PDA) medium and it was spread uniformly by rotating the plates clockwise and anticlockwise direction. Once the media is solidified, then mycelial agar discs (5 mm diameter) from 5 days old actively growing fungal cultures were placed in middle of the petri plates containing PDA with different concentrations of silkworm pupal residue bio soft descent. Similarly, 5 mm mycelial discs of each fungal pathogen placed on the PDA plates without silkworm pupal residue bio soft descent as control. All the plates were incubated at 28 °C and radial growth of mycelia was recorded after 3 to 5 days after incubation. The sensitivity of the fungal species to the silkworm pupal residue bio soft descent was determined by measuring the sizes of radial growth of

mycelia on the agar surface around the disc. All the experiments were carried out in triplicates. Percentage of growth inhibition (GI) was calculated using the formula:

GI (%) = [(Co – CF) / Co] x 100 %

Where, Co is the radial growth of fungal mycelium (control) while, CF is the radial growth of fungal mycelium after inhibition.

g. Evaluation of silkworm pupal residue bio soft descent against early leaf spot (*Alternaria solani)* of tomato under glasshouse conditions

The culture developed on Potato Dextrose Agar (PDA) was used to prove the pathogenicity of the fungal pathogen on tomato plant. 'Koch postulates' was employed for proving the pathogenicity of fungal pathogen by artificial inoculation method under glass house condition. The standard spore suspension was utilized for artificial inoculation on tomato saplings in glass house conditions. Tomato saplings were grown in sterilized soil. Fifteen days old saplings were sprayed with sterile distilled water before inoculation, and then sprayed with spores and mycelial suspension. Inoculated plants were covered with polyethylene bags for 24 hours. After 24 hours of incubation polyethylene bags were removed and kept for the development of disease. The observation on development of symptoms on the leaf was recorded. Silkworm pupal residue bio soft descent was sprayed at different concentrations with high volume sprayer fitted with hollow cone nozzle in order to inhibit the growth of pathogens. The disease severity was recorded at 45, 60, 75 and 90 days.

Three plants were selected randomly in each replication and the observation on disease severity on the foliage was recorded by using 0-5 scale (Mayee and Datar, 1986) and per cent disease severity (PDS) was calculated by using the formula suggested by Wheeler (1969).

$$PDS = \frac{\text{Sum of the individual disease ratings}}{\text{Number of head/leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

Results and Discussion

Studies on development of protocol for extraction of silkworm pupal residue bio soft descent (SPRBD) from silkworm pupal residue revealed that, significantly highest quantity of 2.61 Kg (52 %) of silkworm pupal residue bio soft descent was extracted at 18 tonnes of pressure followed by 20 tonnes pressure 2.20 Kg (44 %). The lowest quantity of silkworm pupal residue bio soft descent (SPRBD) of 1.66 Kg (33 %) was extracted at 16 tonnes pressure (Table 1). At 18 tonnes of pressure about 52 per cent of silkworm pupal residue bio soft descent was obtained compared to the other treatments. Hence, by considering the quantity of bio soft descent extracted, it was opined that pressing of silkworm pupal residue at 18 tonnes of pressure seems to be ideal for getting highest quantity of silkworm pupal residue bio soft descent (SPRBD). The above study seems to be first of its kind.

Table 1

Influence of pressure in extracting SPRBD

Pressure (Tonnes)	Amount of SPRBD (Kg)	SPRBD(%)
16	1.66	33
18	2.61	52
20	2.20	44
F-test	**	-
S. Em±	0.07	-
CD (0.01 %)	0.29	-
CV %	0.10	-

**Significant at 1 %

SPRBD- Silkworm pupal residue bio soft descent

The studies on physical properties of silkworm pupal residue bio soft descent (SPRBD) exhibited creamish color with badam drink odor and slightly turbid in nature.

The microbial load of silkworm pupal residue bio soft descent at 10^{-1} dilution recorded highest bacterial count of 40 CFU/ml followed by actinomycetes (5 CFU/ml) and yeast (3 CFU/ml). However, no fungi were

registered in silkworm pupal residue bio soft descent (Table 2). The absence of fungal load and least microbal load in silkworm pupal residue bio soft descentmay be due to its antimicrobial property which inhibits the growth and development of microbes. These results are comparable with the findings of Priyadarshini (2016) who reported that silkworm pupal chitosan exhibited the antimicrobial property.

TABLE 2

Comparative microbial load in Silkworm pupal residue bio soft Descent

Microorganisms	Number at 10^{-1} dilution (CFU/ml)
Bacteria	40
Fungi	0
Yeast	3
Actinomycetes	5

CFU: Colony forming units

Biochemical composition of silkworm pupal residue bio soft descent (SPRBD)

The results on biochemical analysis of silkworm pupal residue bio soft descent noticed that, the moisture percentage was 8.6, the total protein was 60.77 per cent, the carbohydrate was 5.65 per cent, the total fat was 9.20 per cent, the ash content was 2.27 per cent, the glucose was 1.25 per cent and vitamin E was 1.56 mg, respectively (Table 3).

TABLE 3

Biochemical composition of silkworm pupal residue bio soft descent (SPRBD)

Biochemical composition	Silkworm pupal residue bio soft descent (SPRBD)
Moisture (%)	8.6
Total protein (%)	60.77
Carbohydrates (%)	5.63
Total fat (%)	9.20
Ash (%)	2.27
Glucose (%)	1.25
Vitamin E (mg)	1.56

The results on biochemical composition of silkworm pupal residue bio soft descent (SPRBD) were analyzed for moisture percentage, total protein, carbohydrate, total fat, ash, glucose and vitamin E. The similar kind of findings were witnessed by Kanika *et al.* (2008), Tomotake *et al.* (2010) and Priyadarshini and Revanasiddaiah (2013) who recorded biochemical composition of silkworm pupawere in the range of 55.6 to 81.60 per cent, 0.47 to 6 per cent,1.80 to 8.23 per cent, 2.1 to 4.3 per cent, 2.69 to 5.48 per cent and 23. 2 (ME per kg) for protein content, fat content, carbohydrates, ash content, vitamins and energy respectively. But, these findings were with respect to the biochemical composition of raw silkworm pupae. Analysis of biochemical composition in silkworm pupal residue biosoft descent was done for the first time. However, the silkworm pupal residue bio soft descent was extracted from the silkworm pupae itself and hence the biochemical composition of SPRBD was compared with the silkworm pupae.

Chemical composition of silkworm pupal residue bio soft descent

The results on chemical composition of silkworm pupal residue bio soft descent exhibited that, the pH was neutral (6.8), 1.36 dsm^{-1} of electrical conductivity and the organic carbon per cent was 18 per cent. The major nutrients *viz.,* the nitrogen (N) per cent was 3.41 per cent, the phosphorous (P) per cent was 0.95 per cent and the potassium (K) per cent was 0.59 per cent, respectively. The secondary nutrients like calcium, magnesium and sulphur were 0.32 per cent, 0.29 per cent and 0.17 per cent respectively. The minor nutrients *viz.,* sodium (Na), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and boran (B) were 0.07 per cent, 119.33 ppm, 2.97 ppm, 176 ppm, 5.13 ppm and 1.36 ppm, respectively (Table 4).

The chemical composition of silkworm pupal residue bio soft descent, which is interpreted in the results above are comparable with the investigations of Heenkende and Parama (2010) who disclosed the chemical compositions of silkworm pupae for both the major and minor nutrients like nitrogen (4-12 %), phosphorous (0.58-1.82 %), potassium (0.80 – 1.25%), calcium (0.29-0.65%), magnesium (0.22 to 0.46%),

TABLE 4
Chemical composition of silkworm pupal residue bio soft descent (SPRBD)

Chemical composition	Silkworm pupal residue bio soft descent(SPRBD)
pH	6.8
EC(dsm^{-1})	1.36
Organic carbon (%)	18
Nitrogen (%)	3.41
Phosphorus (%)	0.95
Potassium (%)	0.59
Calcium (%)	0.32
Magnesium (%)	0.29
Sulphur (%)	0.17
Na (%)	0.07
Zn (ppm)	119.33
Cu (ppm)	2.97
Fe (ppm)	176
Mn (ppm)	5.13
B (ppm)	1.36

sodium (0.25 -0.38%), iron (0.2-0.51%), manganese (0.1 -0.3%) and zinc (0.17-0.2%). All the nutrients present in silkworm pupae are mainly obtained from consumption of mulberry leaves which contain both the biochemical constitutions and nutrients in required proportion. Hence, the silkworm pupae might have derived the nutrients from the mulberry leaf, are stored in the pupae during the developmental process. However, the nutrient analysis and composition of nutrients varies on the processing methods of pupae like green pupae, dried pupae and powdered pupae etc.

The present study demonstrated that, the silkworm pupal residue bio soft descent was extracted without electric power can be utilized as a plant nutrient source and as an anti microbial agent in biocontrol. The remained pupal residue after extraction of silkworm pupal residue bio soft descent can be further dried with less power consumption for value addition. The pupal residue after extraction of bio soft descent can be used in making delicious and nutritious food products like cookies, chocolates and nutribar etc. as value addition. So that, conversion of waste into biological useful product. 'Best Out of Waste'is achieved. Due to value addition the reelers can expect higher returns through effective utilization of silkworm pupal residue. By this, the economic status of highly deprived reelers category in sericulture industry can be uplifted.

In vitro evaluation of silkworm pupal residue bio soft decent (SPRBD) against fungal pathogens

The efficacy of silkworm pupal residue bio soft decent (SPRBD) was evaluated at six concentrations *viz.,* 5, 10, 20, 30 , 40 and 50 per cent against different fungal pathogens usually occurring on solonacious vegetable crops. The air borne pathogen *Alternaria solani* and soil born pathogens *viz.,* are *Fusarium oxysporium, Pythium* spp., *Rhizoctania soloni* and *Sclerotium rolfsii* were tested by using SPRBD. The radial growth of the mycelium was recorded at seven days after inoculation.

The silkworm pupal residue bio soft descent (SPRBD) was found to inhibit the growth of all the fungal pathogens with increasing concentration in the medium. Higher mycelial inhibitions (more than 50 %) were observed at 20 per cent concentration of silkworm pupal residue bio soft descent (SPRBD). Interestingly, at 50 per cent concentrations of silkworm pupal residue bio soft descent (SPRBD) cent percent inhibition of mycelial growth was observed in *Alternaria solani* as well as in *Fusarium oxysporium* (Table 5).

Among different concentrations of silkworm pupal residue bio soft descent (SPRBD) 5 per cent concentration showed more than 60 per cent inhibition for *Alternaria solani* (64.71%) and *Fusarium oxysporium* (60 %) respectively. While at 5 per cent concentration the inhibition of 30 per cent was noticed for *Rhizoctania soloni,* 30.77 per cent of inhibition was observed in case of *Pythium* spp. and 7.41 per cent inhibition was recorded for *Sclerotium rolfsii,* respectively (Table 5).

TABLE 5

In vitro evaluation of silkworm pupal residue biosoft descent (SPRBD) against different fungal pathogens

Concentration of SPRBD	Per cent inhibition over control				
	Alternaria solani	Fusarium oxysporium	Rhizoctonia solani	Pythiumspp.	Sclerotium rolfsii
T_1:5 %	64.71	60.00	30.00	30.77	7.41
T_2:10 %	76.47	66.67	50.00	50.00	44.44
T_3:20 %	88.24	75.00	60.00	71.15	62.96
T_4:30 %	94.12	83.33	76.00	76.92	72.22
T_5:40 %	100.00	90.00	84.00	80.77	81.48
T_6:50 %	100.00	100	92.00	88.46	88.89
Mean	87.26	78.17	65.33	66.34	60.02
F-test	*	*	*	*	*
S. Em±	2.587	3.218	1.944	2.58	3.21
CD at 5 %	7.971	9.916	5.989	7.97	9.91

*- Significant at 5 %

At 10 per cent concentration of silkworm pupal residue bio soft descent (SPRBD) significantly highest inhibition of mycelial growth of 76.47 per cent was observed in *Alternaria solani* followed by 66.67 per cent inhibition was exhibited for *Fusarium oxysporium.* While, 50 per cent inhibition of mycelial growth was recorded for *Rhizoctania soloni* and *Pythium* spp. Whereas, the lowest inhibition of mycelial growth of 44.44 per cent was seen in case of *Sclerotium rolfsii* (Table 5).

Significant difference was found among all the tested fungal pathogens at 20 per cent concentration of silkworm pupal residue bio soft descent (SPRBD). Highest per cent inhibition of mycelial growth of 88.24 per cent was found in *Alternaria solani* followed by 75 per cent inhibition in case of *Fusarium oxysporium.* Whereas, 71.15 per cent inhibition of mycelial growth was observed for *Pythium* spp., inhibition of mycelial growth of 62.96 per cent was recorded in *Sclerotium rolfsii* and lowest inhibition of mycelial growth of 60 per cent was observed in *Rhizoctania soloni* (Table 5).

At 30 per cent concentration of silkworm pupal residue bio soft descent (SPRBD) maximum of 94.12 per cent inhibition of mycelial growth was documented in *Alternaria solani* followed by 83.33 per cent inhibition of mycelial growth for *Fusarium oxysporium.* Whereas, 76.92 per cent of inhibition was recorded in case of *Pythium* spp., 76 per cent inhibition was observed for *Rhizoctania solani* and minimum of 72.22 per cent inhibition of mycelial growth was exhibited in *Sclerotium rolfsii* (Table 5).

Similarly, at 40 per cent of silkworm pupal residue bio soft descent (SPRBD) significantly highest inhibition of 100 per cent mycelial growth was documented in *Alternaria solani* followed by 90.00 per cent inhibition of mycelial growth for *Fusarium oxysporium.* Whereas, 84 per cent inhibition was observed for *Rhizoctania solani,* 81.48 per cent inhibition of mycelial growth was exhibited in *Sclerotium rolfsii* and lowest inhibition of 80.77 per cent was recorded in case of *Pythium* spp. (Table 5).

Interestingly, at 50 per cent of silkworm pupal residue bio soft descent (SPRBD) 100 per cent inhibition of

mycelial growth was noticed for *Alternaria solani* as well as for *Fusarium oxysporium.* However, 92 per cent inhibition of mycelial growth was witnessed for *Rhizoctania solani* followed by 88.89 per cent inhibition of mycelial growth for *Sclerotium rolfsii* and lowest inhibition of mycelial growth of 88.46 per cent was recorded for *Pythium* spp. (Table 5).

The *in vitro* evaluation of silkworm pupal residue bio soft descent against fungal pathogens is first of its kind. The inhibition of the fungal pathogens might be mainly due the presence of various antimicrobial peptides cercopin B, D, moricin and also immune proteins which inhibited the growth and development of mycelia proving it as best due to 100 per cent inhibition against almost all the pathogens at increased concentration. This implied that, it can be promisingly used in the control of diseases.

Antimicrobial activity of silkworm pupal residue bio soft descent was in positive support with the findings of Chen *et al.* (2016) who revealed that, the prophenoloxidase (proPO), immune deficiency (IMD) and Toll are the major signaling pathways leading to melanization and antimicrobial peptide production. Peptidoglycon recognition protiens (PGRPs) act as receptors and negative regulators in these pathways and some PGRPs exhibit antimicrobial activity. Chen *et al.* (2014) also revealed that, it also acts as bactericide and fungicide via its amidase activity. Small silk proteins of the domesticated silkworm, *Bombyx mori*, called seroins, act as antimicrobial agents due to involvement of these proteins in the inhibition of pathogens. Binding competition assays followed by antimicrobial assays showed that seroins bind to peptidoglycan, a cell wall component of bacteria (Singh *et al.,* 2014). The antimicrobial protein cercopin B as well as moricin and immune proteins *viz.,* P4, P5 and cercopin D isolated from silkworm pupae rapidly induced the development of several pathogens. Moreover, other proteins were identified in the cocoon silk apart from fibrion and sericin, many of which are immune related proteins. A total of 129 proteins were identified, 30 of which were annotated as protease inhibitors. These protease inhibitors have many intra molecular disulfide bonds to maintain their stable structure and remained active after being boiled. The extracted proteins from the silkworm cocoon found that they had a strong inhibitory activity against fungal proteases. They also reported that extracted cocoon proteins inhibited the germination of *Beauveria bassiana* spores under *in vitro* conditions. The present study added a new understanding to the antimicrobial function (Guo *et al.,* 2016).

The kind of study by using the deacetylated chitin from silkworm pupae was revealed for inhibiting the growth and development of gram negative (*Escherichia coli*) and gram positive bacteria (*Bacillus thuringiensis*, *Staphylococcus aureus,* and *Enterococcus faecalis*). Among the different concentrations 750 µl / ml showed maximum inhibition against the pathogens (Priyadarshini, 2016).

Evaluation of silkworm pupal residue bio soft descent (SPRBD) under glass house conditions against early leaf spot disease (*Alternaria solani)* of tomato

The experiment was carried out in glass house to know the effect of silkworm pupal residue bio soft descent (SPRBD) against *Alternaria solani* occurring on tomato crop. The experiment was carried out with eight treatments and in triplicates. The disease severity was recorded at different growth stages of the crop.

Among different treatments, the minimum disease severity of 6.70 per cent was recorded from the plants treated with silkworm pupal residue bio soft descent (SPRBD) at 10 per cent before and after appearance of disease (T_6) at 45 days followed by the plants treated with recommended fungicide mancozeb (T_7) (7.00 %). The maximum disease severity of 11 per cent was observed in the untreated plants (control-T_8) (Table 6).

At 60 days, significantly the minimum severity of 14.33 per cent was noticed in the plants treated with with silkworm pupal residue bio soft descent (SPRBD) at 10 per cent before and after incidence of disease (T_6) followed by the plants treated with recommended fungicide mancozeb (T_7) (14.83 %). The maximum disease severity of 29.17 per cent was seen in the untreated plants (T_8) (Table 6).

TABLE 6

Effect of silkworm pupal residue bio soft descent (SPRBD) on disease severity of *Alternaria solani* of tomato under glass house conditions

Treatments	Per cent Disease Severity			
	45 Days	60 Days	75 Days	90 Days
T_1: SPRBD @ 5 % spray before inoculation with Alternaria solani	7.80	17.00	35.27	51.37
T_2:SPRBD @ 5 % spray after inoculation with Alternaria solani	8.10	19.03	37.67	51.67
T_3:SPRBD@ 5 % spray before and after inoculation with Alternaria solani	7.03	16.33	32.67	47.27
T_4:SPRBD@ 10 % spray before inoculation with Alternaria solani	7.13	15.33	34.47	49.13
T_5:SPRBD@ 10 % spray after inoculation with Alternaria solani	7.50	16.17	36.17	51.07
T_6:SPRBD@ 10 % spray before and after with Alternaria solani inoculation	6.70	14.33	28.93	43.10
T_7:Recommended fungicide (Mancozeb)	7.00	14.83	31.33	48.67
T_8: Control (Untreated)	11.00	29.17	49.00	75.40
Mean	7.78	17.78	35.69	52.21
F-test	*	*	*	*
S. Em±	0.35	1.32	1.30	1.75
CD at 5 %	1.05	3.98	3.89	5.26

*- Significant at 5 %

Similarly, the minimum disease severity of 28.93 per cent was exhibited in the plants treated with silkworm pupal residue bio soft descent (SPRBD) at 10 per cent before and after incidence of disease (T_6) followed by the plants treated with recommended fungicide mancozeb (T_7) (31.33 %). However, the maximum disease severity of 49.00 per cent was observed in the untreated plants (T_8) at 75 days (Table 6).

At 90 days significantly the minimum disease severity of 43.10 per cent was exhibited in the plants treated with silkworm pupal residue bio soft descent (SPRBD) at 10 per cent before and after incidence of disease (T_6) followed by the plants treated recommended fungicide mancozeb (T_7) (48.67 %). Whereas, the maximum disease severity of 75.40 per cent was observed in the untreated plants (T_8) at 90 days respectively (Table 6).

From the above results, it was concluded that all the treated plants recorded significantly less per cent severity of the disease at 5 and 10 per cent concentration of SPRBD.

Due to increasing awareness of environment pollution by way of continuous use of chemical pesticides and their residual toxicity on crops like effort are being made to use some eco-friendly substitutes like plant extracts. In view of their non phytotoxicity and systemic actions, they have gained the attention. Several plant extracts known to possess antifungal activities are being exploited to manage fungal plant diseases. Like the plant extracts the organic form of silkworm pupal residue bio soft descent has given the better results in inhibiting the tested pathogens under *invitro* conditions. So, this might be used as biocontrol or organic extract in controlling many diseases due to its antimicrobial nature.

The *in vitro* evaluation of silkworm pupal residue bio soft descent against *Alternaria solani*, *Fusarium oxysporium, Pythium* spp., *Rhizoctania stolonifer*

and *Sclerotium rolfsii* being the first kind of work and hence inhibition of these pathogens by botonicals and bioagents were compared. Nashwa and Abo-Elyousr (2012) reported that the leaf extracts of *Datur astramonium, Azadirachta indica* (Neem)and *Allium sativum* (Garlic) at 5 per cent concentration caused the highest reduction of mycelial growth of *A. solani* (44.4, 43.3 and 42.2% respectively).

Biopesticides extracted from plants like *Withania somuifera, Lawsonia inermis* and *Datura stramonium* completely inhibited the growth of *Fusarium oxysporum* at 50 per cent concentration both under *in vitro* and *in vivo* conditions (Khandare and Salve, 2011). Adhikari *et al.* (2013) reported that among 70 isolates, 21 antagonistic isolates representing biovars of *Pseudomonas fluorescens* (biovars I, II, III, and V) collected from the rhizosphere were found to act as an antifungal agent under in vitro conditions against *Rhizoctonia solani*.

Use of silkworm pupal residue bio soft descent is a potential, non-chemical means of controlling plant diseases by reducing inoculum levels of pathogens. Such a management would help in preventing the pollution and also health hazards. Use of silkworm pupal residue bio soft descent for control of diseases leads to the proper utilization of silkworm pupal residue as byproduct generated in large quantities at silk reeling units which otherwise go as waste.

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