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EVALUATION OF BIOFORMULATION FOR THE MANAGEMENT OF GUMMY STEM BLIGHT DISEASE IN MUSKMELON

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ABSTRACT: *Penicillium verruculosum* (KU645999) isolated from rhizospheric soil of healthy muskmelon plants was used for bio formulation along with the potential crude oligosaccharide elicitor extracted from its cell wall. Their ability was evaluated for the control of gummy stem blight disease of muskmelon under greenhouse condition. Different carrier materials (Talc, Rice Bran and Wheat bran) were tested for the survival of the fungus up to 90 days and found that talc based formulation was very efficient carrier material for the fungal population survival among all the carrier materials tested. The bio formulation was evaluated for disease protection against *Stagonosporopsi scucurbitacearum*(KJ782214)in muskmelon after challenge inoculation by the pathogen. The results showed that talc based formulation was best in disease protection induction with 81.62% followed by rice bran 73.05% and 70.05% by wheat bran compared to positive control of 93.42%. Present study reveals that talc based bio formulation of *Penicillium verruculosum* supplemented with its crude oligosaccharide elicitor enhances the resistance to gummy stem blight disease of muskmelon under greenhouse conditions.

KEYWORDS: *Penicillium verruculosum*, *Stagonosporopsis cucurbitacearum,* crude oligosacchar ide, gummy stem blight, bioformulation.

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1.INTRODUCTION

Rhizosphere soil holds the best biological control agents against various plant pathogens. They not only protect the plants from the attack by pathogens but also supports growth of plants by allowing

them to utilize wide range of organic compounds from soil. *Trichoderma, Penicillium, Bacillus, Pseudomonas* etc. are few major biocontrol agents widely used in the management of soil borne plant pathogens [1-3]. They are found to work their best regarding biocontrol when applied in combinations of two or more strains of fungi and bacteria or both than when applied alone [4-6]. A significant number of organic compounds including secretions, sloughed off cells, lysates and exudates are secreted by actively growing roots into the rhizosphere [7]. Bioformulation is even more effective when combine the biocontrol agent along with the organic compounds and elicitors in reduction of plant disease. *Trichoderma*spp. when formulated with chitosan found to reduce the club weight on root significantly, but did not increase the top weight of the plants [8]. An antifungal compound was isolated from *Epicoccumpurpurescens* and found its fungi toxic nature against *S. sclerotiorum*in sunflower [9]. Chitin is an abundant renewable natural resource obtained from manne invertebrates, insects, fungi and algae [10]. Application of biocontrol agents along with chitin has greater importance in plant disease management [11-12]. The biocontrol agents are specific to their target, effective and also economical compared to chemical fertilizers. They are environmental friendly and plays major role in sustainable agriculture. In the present study effective bio control agent *P. verruculosum* and its crude oligosaccharide elicitors which are previously found effective individually for plant growth promotion and reduction of gummy stem blight disease in muskmelon were tested for their efficacy as bio formulation. The study also emphasizes on the screening of carrier based formulation which can support the growth and survival of fungi is useful to maintain their viability in the environment which helps the effectiveness of bio control ability. This mixture of bio formulation can be an efficient alternative against *S. cucurbitacearum* in muskmelon plants.

2. MATERIALS AND METHODS

2.1 Isolation of GSB pathogen (*S. cucurbitacearum*DBTNP 2) and rhizospheric fungus (*P. verruculosum*MRS-PGPF 24)

The pathogen was isolated from infected muskmelon samples collected from fields around Karnataka by standard blotter method. The rhizospheric fungi was isolated from soil samples of healthy muskmelon rhizospheric soil samples by serial dilution method. The pure cultures of pathogen and rhizospheric fungi obtained were identified individually on the basis of macroscopic (culture morphology and appearance) and microscopic (mycelia and conidia) characteristics [13].

2.2 Preparation of GSB pathogen (*S. cucurbitacearum*DBTNP 2) and rhizospheric fungal (*P. verruculosum*MRS-PGPF 24) inoculums

S. cucurbitacearum and rhizospheric fungus were mass cultured on Potato Dextrose Agar (PDA) plates and incubated at 25 ± 2° C for 7 days. Spore suspension was prepared from freshly sporulating cultures (7- day- old) by flooding the cultures with Sterile Distilled Water (SDW) by scraping the surface of PDA plates with sterile spatula. The concentration of the inoculum was adjusted to 1×10^5cfu ml^{-1} for *S. cucurbitacearum* and 1×10^8cfu ml^{-1} for *P. verruculosum*using Haemocytometer

and used for further studies [14-15].

2.3 Preparation of rhizospheric fungus (*P. verruculosum*MRS-PGPF 24) and crude oligosaccharide

Therhizosphericfungus*P. verruculosum*MRS-PGPF 24was screened in our previous experiments for its plant growth promoting traits as well as antifungal activity against *S. cucurbitacearum*DBTNP 2the gummy stem blight causing agent in muskmelon. The fungus was mass multiplied in potato dextrose broth. The sterilized broth (121° C, 15 psi for 30 min) was inoculatedwith actively growing mycelial disc of nine mm diameter and incubated for 15 days. Crude Oligosaccharide was extracted from the driedmycelial mat of the PGPF (MRS-PGPF 24) and resultant extract was lyophilized [16]. CO powder was diluted with SDW to prepare 4 mg ml^{-1}concentration and used for seed treatment for 6h. For combined treatment, elicitor and fungal mixture was prepared by adding the crude oligosaccharide at 4 mg ml^{-1} concentration added to broth (1 % v/v) and used for the preparation of bio formulation.

2.4 Preparation of carrier material of Talc-based, Rice bran and Wheat bran- for inoculation of rhizosphericfungal (*P. verruculosum* MRS-PGPF 24) inoculum and crude oligosaccharide

Powdered talc, rice bran and wheat bran were chosen as carriers. They were steam sterilized at 140 kPa for 30 min, and dried aseptically in trays for 12 h at 50° C before use. The fungal biomass along with the medium and elicitor mixture was incorporated into the different sterilized carriers at 50 ml suspension per 100 g and thoroughly mixed with addition of 500 mg CMC [17]. All the formulations were prepared in the same manner but without combined inducers amended with CMC served as negative control and with Mancozeb served as positive control. The product was shade dried to decrease the moisture content (less than 20%), then packed in polypropylene bags and sealed and stored in room temperature with less than 35% moisture content [18].

Table 1: Preparation of Bio Formulation

Carrier Material	Treatment	Negative control	Positive control
Talc (1 kg) + CMC (10 g)	Suspension containing *P. verruculosum*MRS-PGPF 24 (1×10^8cfu ml^{-1}) + CO (4 mg ml^{-1})	Without combined inducers amended with CMC	Without combined inducers amended with Mancozeb
Rice bran (1 kg) + CMC (10 g)			
Wheat bran (1 kg) + CMC (10 g)			

2.5 Screening of carrier material Talc-based, Rice bran and Wheat bran- inoculums for survival of rhizosphericfungus (*P. verruculosum* MRS-PGPF 24)

Carrier based bio formulation samples inoculated with mixture of rhizospheric fungus and crude oligosaccharide were drawn at periodical intervals and population was assessed by serial dilution technique.

2.6 Efficacy of bio formulations against GSB disease protection under green house conditions

The efficacy of treatment with *P. verruculosum*MRS-PGPF 24 + CO various carrier based formulations for control of GSB disease of muskmelon was evaluated under green house conditions. The first true leaf of muskmelon plants raised from seeds pretreated with bioformulation, positive and negative controls were challenge inoculated with suspension of pathogen. The pots were then arranged in a randomized complete block design and maintained under greenhouse conditions. The inoculated plants were covered with plastic bags for two days to maintain humidity and to avoid cross contamination. To evaluate the efficacy of combined inducer, per cent of disease was recorded periodically (every 7 days) and the final count was made 21 days after sowing [15]. The experiment consisted of 4 plants per pot and repeated thrice.

$$\textbf{Percent Disease Protection} = \frac{\mathbf{C - T}}{\mathbf{C}} \times \mathbf{100}$$

Where, C represents percentage (%) of gummy stem blight disease incidence in the control and T represents percentage (%) of gummy stem blight disease incidence in induced plants.

2.6 Statistical analysis

Each experimental data was subjected to analysis of variance (ANOVA) using SPSS Inc. 16.0. Significant effects of treatments were determined by the magnitude of the F value ($p \leq 0.05$). Treatment means were separated by Tukey's HSD test.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Screening of carrier material of Talc-based, Rice bran and Wheat bran- inoculums for survival of rhizosphericfungus (*P. verruculosum* MRS-PGPF 24)

Among various carriers tested, talc based formulation was found to be best for the survival of rhizospheric fungus. The fungus survived with required Colony Forming Units (1 X 10^8cfuml^{-1}) in talc formulation up to 60 Days After Storage (DAS) and after that it declined while the decrease was much earlier in other carrier materials (Table 2).

Table 2: Effect of different carriers on survival of rhizosphericfungus (*P. verruculosum* MRS-PGPF 24)

Treatment	Population of rhizospheric fungus(*P. verruculosum* MRS-PGPF 24) at Days After Storage (DAS) in cfu		
	30 DAS	60 DAS	90 DAS
Talc	2.86± 0.043[a]	3.31±0.037[a]	2.95±0.025[a]
Rice bran	2.64±0.018[b]	3.17±0.019[b]	2.83±0.021[b]
Wheat bran	2.37±0.018[c]	2.49± 0.025[c]	2.37±0.030[c]

Values are means of four independent replicates. ± indicate standard errors. Means followed by the

same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD

3.1.2 Efficacy of bio formulations against GSB protection under green house conditions

The disease protection studies was evaluated under green house conditions with all the three carrier material based formulations (talc, rice bran and wheat bran) along with the controls (SDW treated negative control and Mancozeb treated positive control). The muskmelon plants 21 days after sowing, the seeds treated with talc offered the disease protection of GSB disease by 81.62% followed by rice bran 73.05% and 70.05% by wheat bran compared to positive control of 93.42% (Fig. 1 and 2).

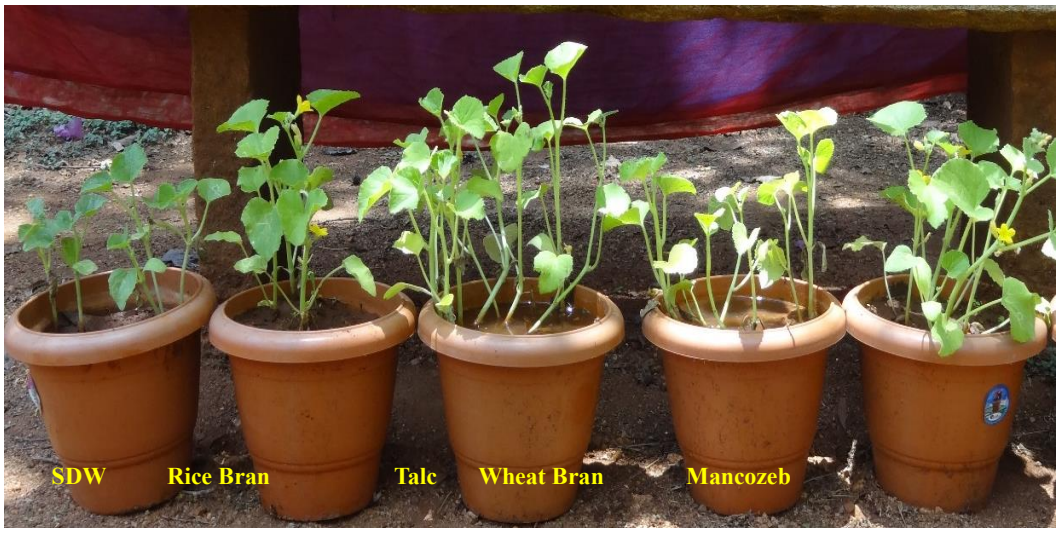

Fig 1: Efficacy of bio formulations against gummy stem blight disease protection under green house conditions by counting the number of emerging seedlings

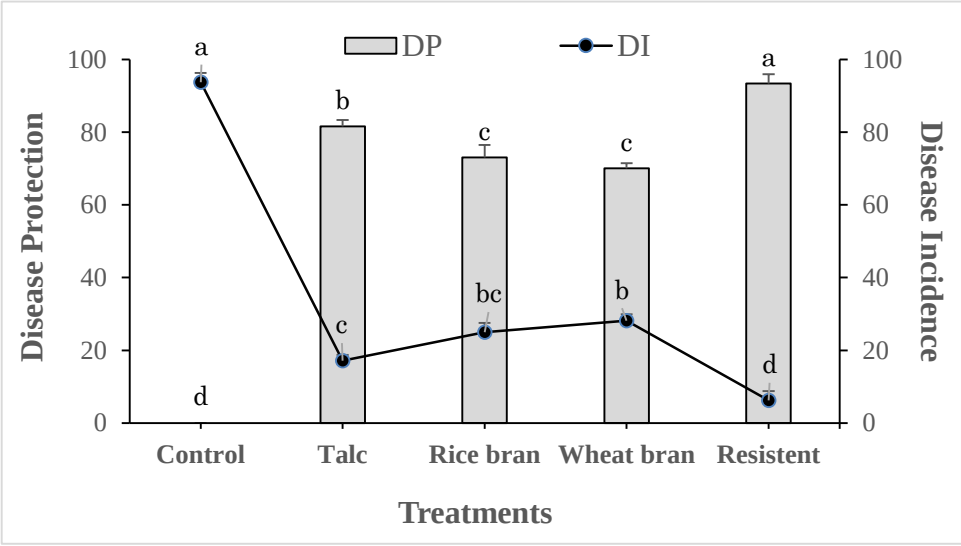

Fig 2: Efficacy of bio formulations against gummy stem blight disease protection under green house conditions by counting the number of emerging seedlings. Vertical bars indicate ± SE. Means followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD

3.2 DISCUSSION

Bioformulations that contain beneficial rhizospheric fungi either one or more strains or along with the metabolites produced by them which is useful to increase plant growth, soil fertility and suppression of phytopathogens. In the present study a bio formulation was prepared using the rhizospheric fungus*P. verruculosum*MRS-PGPF 24 along with its cell wall crude oligosaccharide elicitors. These two were individually studied primarily for its efficacy on their disease protection and also growth promoting ability in muskmelon against gummy stem blight disease. Three different carrier based formulations (talc, rice bran and wheat bran) were screened along with carrier binder CMC for treatment with suspension containing *P. verruculosum*MRS-PGPF 24 (1×10^8cfu ml^{-1}**)** + CO (4 mg ml^{-1}). The survival of fungal population was tested in these carrier materials for 30 days' interval up to 90 days. Talc based formulation was found to be suitable carrier for formulation preparation among all the test samples. Similar studies [19] where reported that among the carrier materials tested, talc retained maximum population of *Trichoderma* spp. and *P. fluorescens* throughout the incubation period. A study was carried out to develop some new bioformulations and evaluate their efficacy in promoting cotton seedlings growth characteristics [20]. For development of formulations, preparation of mineral carriers for increasing stability in interaction between associated PGPR and cotton plants, preparation of bacterial suspension and development of bio formulations used a talc-based powder and bentonite-based powder respectively enhanced the survivability of the microbial strains for a longer period.The addition of microbial or plant produced secondary metabolites to bio formulations may increase agricultural productivity, improving the performance of the inoculation. The use of some secondary metabolites (flavonoids, lipochitooligosaccharides, phytohormones, etc.) in bio formulations that improve leguminous crop yields was studied [21]. Their work reports suggest that the addition of these molecules may contribute to the sustainable development of new agronomic products. In another study the bioformulation mixtures of TvMNT7 with Pfl, TvO with TvOL and TvOwithTvOL and Th along with chitin effectively reduced the incidence of club root-root knot, damping off-root knot, and head rot-root knot respectively in cabbage and cauliflower under green house conditions [19]. Fluorescent *Pseudomonads* control *Pythium* spp. through different modes of action such as competition for nutrients and space [22] antibiosis [23], production of siderophores[24] and lytic enzymes [25]. In addition, induction of resistance by fluorescent *Pseudomonads* is an additional mechanism by which these bacteria protect plants against several diseases [26-29]. *Trichoderma* as a potent fungal biocontrol agent against a range of plant pathogens has attracted considerable scientific attention [30- 33]. Various substrates are being used for the mass multiplication of *Trichoderma* species [34-37].Similarly, in the present study the bio formulation mixture was found to be effective in protection of muskmelon plants challenge inoculated with *S. cucurbitacearum*DBTNP 2, the gummy stem blight causing agent under green house conditions. Talc based formulation resulted in

81.62% disease protection followed by rice bran 73.05% and 70.05% by wheat bran compared to positive control of 93.42% which was treated with the chemical fertilizer (Mancozeb). Even though comparatively mancozeb was more efficient than our formulation mixture it is evident that bio formulation mixture can stand against the chemical fertilizer to an extent where it can also induce growth promotion of plant and give protection from wide range of pathogens.

4. CONCLUSION

Results of the present study clearly indicate that development of stable formulations mixture of rhizospheric fungus along with its crude oligosaccharide elicitors is of great importance and is a promising approach to a sustainable agriculture. The results of the study also indicate that bio formulation mixture may have practical application in biological control of plant disease which can potentially replace the use of chemical fertilizers and pesticides. The use and application of such bioformulation mixture can result in the reduction of application of harmful chemicals, protect the environment and biological resources and can be an important component of integrated pest management that can help the growers to achieve a sustainable agriculture.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

REFERENCES

1. Papavizas GC. Survival of *Trichoderma*and*Gliocladium*: Biology, Ecology and potential for biocontrol. Annual. Review. Phytopathol. 1985; 18: 389-413.
2. Dowling DN, Gara FO. Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Tibtech. 1994; 12: 133-141.
3. Harman, GE. Myths and dogmas of biocontrol. Plant Dis. 2000; 84: 377-393.
4. Paulitz TC, Ahmad IS, Baker, R. Traits of *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol. Rev. 1990; 56: 662-676.
5. Budge SP, McQuilken MP, Fenlon S, Whipps M. Use of *Coniothyriumminitans* and *Gliodadiumvirens* for biological control of *Sclerotiniasclerotiorum* in glasshouse lettuce. Biol. Control. 1995; 5: 513-522.
6. Raupach GS, Kloepper W, Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology. 1998; 88: 1158-1164.
7. Mukherjee G., Dey P., Dhiman S. Agricultural Important Microorganisms: From Rhizosphere to Bioformulation as Biological Control Weapons for Sustainable Agriculture. In: Gehlot P.,

Singh J. (eds) Fungi and their Role in Sustainable Development: Current Perspectives. Springer, Singapore, 2018.

8. Cheah LH, Page BBC. *Trichoderma* spp. for potential biocontrol of clubroot of brassicas. Proc. 50th New Zealand Plant Protection Conference: 1997; 150-153.
9. Pieckenstain FL, Bazzalo ME, Roberts AMI, Ugalde RA. *Epicoccumpurpurascens* for biocontrol of *Sclerotinia* head rot of sunflower. Mycol. Res. 2001; 105: 77-84.
10. Subasinghe S. "The development of crustacean and mollusc industries for chitin and chitosan resources," in: Zakaria MB, Wan Muda WM, Abdullah MP. (Eds.), Chitin and Chitosan, Penerbit University Kebangsaan, Malaysia, pp. 1995; 27-34.
11. Bell AA, Hubbard JC, Liu L, Davis, RM, Subbarao KV. Effects of chitin and chitosan on the incidence and severity of *Fusarium* yellows in celery. Plant Dis. 1998; 82: 322-328.
12. Yuen GY, Steadman JR, Lindgren DT, Schaff D, Jochum C. Bean rust biological control using bacterial agents. Crop Prot. 2001; 20: 395-402.
13. Mathur SB, Kongsdal O. Common laboratory seed health testing methods for detecting fungi. Danish government institutes for developing countries. Copenhagen, Denmark. 2003; 357-364.
14. Raju NS, Niranjana SR, Janardhana GR, Prakash HS, Shekar Shetty H, Mathur SB. Improvement of Seed quality and Weld emergence of *Fusariummoniliforme* infected sorghum seeds using biological agents. J Sci. Food Agric. 1999; 79: 206-212.
15. Zhao J, Quan HX, Guang-Hui S, Lei X, Jia-Li D, Dong-Sheng W. Evaluation of *Streptomyces* spp. for biocontrol of gummy stem blight (*Didymellabryoniae*) and growth promotion of *Cucumismelo* L. BiocontrolSci. Tech. 2012; 22: 23-37.
16. Nita- LM, Heyraud A, Gey C, Braccini I, Lienart Y. Novel oligosaccharides isolated from *Fusariumoxysporum* L. rapidly induce PAL activity in Rubuscells.Acta. Biochim. Pol. 2004; 51: 625-634.
17. Ramakrishnan G, Jeyarajan R, Dinakaran D. Talc based formulation or *Trichodermaviride* for biocontrol of *Macrophomina phaseolina*.1994; 8: 41-44.
18. Vidhyasekaran P, Muthamilan, M. Development of formulation of *Pseudomonas fluorescence* for control of chick pea wilt. Plant Dis. 1995; 79: 782-790.
19. Loganathan, M. Development of bio formulation for the management of Major fungal-nematode complex diseases of Cabbage and Cauliflower in Tamil Nadu. Thesis. Tamil Nadu Agricultural University, Coimbatore, 2002.
20. Ardakani SS, Heydari A, Tayebi L, Cheraghi M. Evaluation of Efficacy of New Bioformulations on Promotion of Cotton Seedlings Growth Characteristics. 2nd International Conference on Environmental Science and Technology IPCBEE. 2011; 6: 361-364.

21. Morel MA, Cagide C, Castro-Sowinski S,Arora N, Mehnaz S, Balestrini R. The Contribution of Secondary Metabolites in the Success of Bioformulations for Sustainable Agriculture. Springer, New Delhi, 2016.
22. Elad Y, Chet I. Possible role of competition for nutrients in biocontrol of damping off by bacteria. Phytopathol.1987;77: 190-195.
23. Howie WJ, Suslow TV. Role of antibiotic biosynthesis in the inhibition of *Pythiumultimum* in cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. Mol. Plant Microbe Interact.1991. 4; 393-399.
24. Loper JE. Role of fluorescent siderophore production in biological control of *P. ultimum* by *P.fluorescens* strain. Phytopathol.1998;78: 166-172.
25. FrindlenderM, Inbar J, Chet I. Biological control of soil borne plant pathogens by a b-1,3glucanase producing *Pseudomonas cepacia*. Soil Biol.Biochem.1993;25: 1211-1221.
26. Zhou T, Paulitz JC. Induced resistance in the biocontrol of *Pythiumaphanidermatum* by *Pseudomonas*spp on cucumber. J Phytopathol.1994; 142: 51-63.
27. Chen C, Belanger RR, Benhamou N, Paulitz, TC. Induced systemic resistance (ISR) by *Pseudomonas* spp. impairs pre and post infection development of *P. aphanidermatum*on cucumber roots. Eur. J. Plant Pathol. 1998; 104: 877-886.
28. Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samiyappan R. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plant against pest and diseases. Crop Prot.2001; 20: 1-11.
29. Zehnder GW, Murphy JF, Sikora EJ, Klopper KW. Application of rhizobacteria for induced resistance. Eur. J. Plant Pathol. 2001; 107: 39-50.
30. Choudhary CS, Jain SC, Ritesh Kumar, Jaipal Singh C. Efficacy of different fungicides, biocides and botanical extract seed treatment for controlling seed-borne *Colletotrichum* sp. in chilli (*Capsicum annuum* L.). The Bioscan, 2013; 8(1):123-126.
31. Santosh RM, Nargund VB, Hegde RV. Management of fruit rot causing seed borne fungal pathogens in chilli. The Bioscan. 2014; 9(1):403-406.
32. Rini CR, Sulochana KK. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctoniasolani* and *Fusariumoxysporum* infecting tomato. J. Tropical Agri. 2007; 45:21-28.
33. Tewari AK, Mukhopadhyay AN. Testing of different formulations of *Gliocladiumvirens* against chickpea wilt complex. Ind. Phytopathol. 2001; 54:67-71.
34. Chakrabarty R, Acharya GC, Sarna TC. Evaluation of substrates for mass multiplication of bioagent*Trichodermaviride*. Afri. J. Agri. Res. 2014; 9:1938-1940.
35. Kumar S, Roy PD, Lal M, Chand G, Singh V. Mass Multiplication and shelf life of *Trichoderma* species using various agroproducts. An Inter. Quarterly J. Life Sci. 2014; 9:1143-1145.

36. Rajput AQ, Khanzada MA, Shahzad S. Effect of different substrates and carbon and nitrogen sources on growth and shelf life of *Trichoderma pseudokoningii*. Inter. J. Agr. Biol. 2014; 16:893-898.
37. Krishna AH, Kumar MR. *In vitro* screening and evaluation of different substrates and carrier materials for mass multiplication of *Trichoderma* against dry root rot in acid lime incited by *Fusariumsolani*. Res. J. Pharma. Biol. and Chem. Sci. 2013; 4:53-60.