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Biocidal Activity Of Spinosad Against Selected Soil Beneficial And Plant Pathogenic Microbes

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Abstract: This research was initiated to investigate the antimicrobial activity of Spinosad (Microbial metabolite) which is a new type of insecticide with natural origin was studied for its activity against selected soil beneficial microbes and plant pathogenic microbes were tested against spinosad for its activity. The activity of spinosad was tested against soil beneficial and plant pathogenic microbes by disc diffusion method. The spinosad was prepared at 1 ppm, 5 ppm & 10 ppm concentrations and tested against bacteria & fungi. The mean and S.D value of soil beneficial bacteria against spinosad at 1ppm concentration was 2±3.46 (95% confidence level), at 5ppm concentration 2.33±4.04,and at 10 ppm concentration the value was 4.66±4.04.the activity of spinosad against soil beneficial bacteria was low when compared to plant pathogenic bacteria when comparing the results, The mean and S.D of plant pathogenic bacteria at 1ppm was (12±7), at 5 ppm (13.66±1.15) and at10 ppm (15±2) so, from this results we have concluded that spinosad is harmless to soil beneficial bacteria and fungi whereas harmful to disease causing Plant pathogenic microbes.

Keywords: Spinosad, Soil Beneficial Microbes, Plant Pathogenic Microbes

INTRODUCTION

Agriculture is the back bone of Indian economy and about 60 % of the population depends on agriculture as their only occupation. To maintain self-sufficiency, the food production should be increased from the current status. Important factors that affect attaining and maintaining the food production are population, land availability and the devastation of agricultural products by the pests. It is estimated that 35 % of the potential production is lost due to pest (Cramer, 1967). Bioinsecticides are currently studied more and more because of the possibility of their use in plant protection as an alternative method to the broad use of conventional pesticides. The development of environmental friendly insecticides, having specificity to insects along with low toxicity to vertebrates, has captured worldwide attention of scientists. Spinosad is a new type of insecticide with natural origin.

A new Saccharopolyspora species isolated from soil collected in sugar mill rum still is described. This organism is characterized by pale yellowish pink aerial hyphae that bear long chains of spores encased in distinctive spiny spore sheaths. Fragmentation occurs when the organism is cultured in liquid media. The name proposed for this new species is Saccharopolyspora spinosa (Mertz & Yao, 1990).

Spinosad represents a new class of fermentation derived tetracyclic macrolides with unique activity against a variety of lepidopterous pests and possesses very favorable mammalian toxicity and environmental profiles. Additionally, Spinosad exhibits a great deal of selectivity towards beneficial insects, especially predators enabling extensive utility in the diamond back moth (DBM) and other integrated pest management (IPM) programs (Crouse and Sparks,1998). Because of various beneficial features of Spinosad, it looks forward to great practical merits and thus having broad marketing prospective.

The previous researchers mentioned that spinosad has unique mode of action which may reduce the probability of being cross resistant to other cholinesterase inhibitor insecticides (Liu et al., 1999).moreover its rapid contact and ingestion activity in insects causing excitation of the nervous system, leading to cessation of feeding and paralysis (Thompson and Hutchins ,1999).

(Naresh et al., 2011) have compared the effect of Spinosad and Neem Seed Kernel Extract as Bio-Controlling agents for Malarial Vector, Anopheles stephensii and Non-Biting Midge, Chironomus circumdatus. The work reveals that the novel pesticide Spinosad seems to be more effective than the NSKE.

MATERIALS AND METHODS:

Collection of the naturally derived insecticide SPINOSAD, Trade name - Tracer was purchased from Bharathi Agro Centre at Athur (Salem District).

Microorganism used:

Collection of plant pathogenic organisms:

The three plant pathogenic bacteria namely Erwinia carotovora, Pseudomonas syringae, Xanthomonas campestris and three plant pathogenic fungi namely

Aspergillus niger, Rhizopus stolonifer and Mucor circinelloides were obtained from Department of Plant Pathology, Annamalai University.

Collection of soil beneficial organisms:

Soil beneficial bacteria viz., Azotobacter chroococcum Pseudomonas fluorescens Rhizobium leguminosarum were isolated from rhizosphere soil. The soil samples were serially diluted up to 10-6 dilution. 1 ml of aliquots of last dilution was plated in appropriate medium and the plates were incubated. The inoculated plates were then examined for the results and fungi viz., Aspergillus fumigatus, Penicillium chrysogenum, Trichoderma viride were collected from the soil sample and were plated in Sabouraud Dextrose Agar. The agar plates were observed for colony morphology and the structural characteristic of the fungi were observed by (LPCB) Lacto phenol cotton blue wet mount.

Biochemical tests:

All the isolates of soil beneficial and plant pathogenic organisms were microbiologically and biochemically characterized for Gram reaction, motility, nitrite reduction, IMViC tests, citrate utilization, catalase test, oxidase test, starch hydrolysis, gelatin liquefaction and different carbon source as per the standard methods.

Cultures maintenance and inoculum preparation

Maintenance of bacterial cultures used in the study

The test bacterial isolates were sub-cultured and maintained on Nutrient agar slants and stored in refrigerator at 4C.

Bacterial inoculums preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards and then used for the determination of antibacterial activity.

Maintenance of fungal cultures used in the study

The test fungal isolates were sub-cultured and maintained on Sabouraud's dextrose agar slants and stored in refrigerator at 4C.

Fungal inoculums preparation

Fungal inoculums was prepared by inoculating a loopful of test organisms in 5 ml of Sabouraud's dextrose broth and incubated at 28C for 2 days (yeasts) and 3 days (moulds) till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards and then used for the determination of antifungal activity.

Antibacterial activity of spinosad using disc diffusion method

The spinosad was prepared at different concentrations such as 1 ppm, 5 ppm & 10 ppm and tested against bacteria and fungi. Six (mm) diameter discs were prepared using sterile Whatmann No.1 filter paper. The antibacterial activity of spinosad was determined by disc diffusion method proposed by Bauer et al. (1966). Petriplates were prepared by pouring 20 ml of Mueller Hinton agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were solidified and 24 hrs fresh bacterial cultures were swabbed uniformly, the plates were allowed to dry for five minutes. After drying, the discs with spinosad were placed on the surface of the plate using sterile forceps and gently pressed to ensure contact with the agar surface. The plates were incubated at 37C for 24 hours. The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

Antifungal activity of spinosad using disc diffusion method

The antifungal activity of spinosad was determined by disc diffusion method proposed by Bauer et al. (1966). Petriplates were prepared by pouring 20 ml of Sabouraud's dextrose agar and allowed to solidify for the use in susceptibility test against fungi. Plates were solidified and 24 hrs fresh cultures were swabbed uniformly, the plates were allowed to dry for five minutes. After drying, the discs with spinosad were placed on the surface of the plate using sterile forceps and gently pressed to ensure contact with the agar surface. The plates were incubated at 28C for 48 hours (yeasts) and 72 hours (molds). The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

RESULTS:

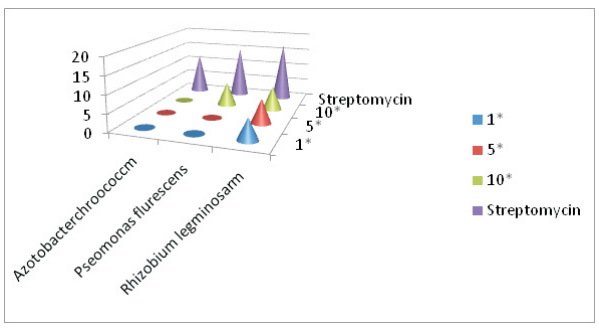
Antimicrobial activity of spinosad against soil beneficial bacteria

The efficacy of spinosad was observed by performing antibacterial activity of spinosad against soil beneficial bacteria. The spinosad disc was prepared at different concentrations viz., 1 ppm, 5 ppm & 10 ppm and were tested against Azotobacter chroococcum, Pseudomonas fluorescens and Rhizobium leguminosarum. Streptomycin was used as a positive control and the results were tabulated (Table-1) and graphical representation in (graph-1).The 10 ppm concentration of spinosad showed minimal inhibition zone of 7 mm against Pseudomonas fluorescens and Rhizobium leguminosarum with minimal inhibition zone of 6 mm, 7 mm and 7 mm with respect to 1 ppm, 5 ppm and 10 ppm.

Table-1: Antimicrobial activity of spinosad against soil beneficial bacteria

Soil Beneficial Bacteria	Inhibition Zone diameters (mm)			Positive control
	Different concentration of spinosad			
	1 *	5 *	10 *	Streptomycin (30 µg/ml)
Azotobacter chroococcum	0	0	0	12
Pseudomonas fluorescens	0	0	7	15
Rhizobium leguminosarum	6	7	7	17
Mean ± S.D	2±3.46	2.33±4.04	4.66±4.04	14.66±2.51
CD (0.05)	8.60	10.04	10.03	6.25

Graph-1: Antimicrobial activity of spinosad against soil beneficial bacteria



* Parts per Million

Antimicrobial activity of spinosad against soil beneficial fungi

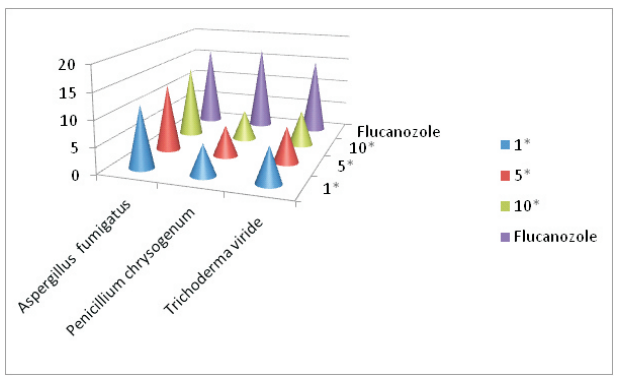
The efficacy of spinosad was observed by performing antimicrobial activity of spinosad against soil beneficial fungi. The spinosad disc was prepared at different concentrations viz., 1 ppm, 5 ppm & 10 ppm and were tested against Aspergillus fumigatus, Penicillium chrysogenum and Trichoderma viride. Flucanozole was used as a positive control and the results were tabulated (Table-2) and graphical representation in (graph-2).The spinosad disc exhibited pronounced inhibition to all organisms. The minimal inhibition was observed in Penicillium chrysogenum with 6 mm with respect to all the three different concentrations, 1 ppm, 5 ppm &10 ppm and Trichoderma viride with minimal inhibition zone of 7 mm, with respect to 1 ppm, 5 ppm & 10 ppm. The maximum inhibition was observed as 12 mm, 13 mm & 14 mm in Aspergillus fumigatus with respect to 1 ppm, 5 ppm & 10 ppm.

Table-2: Antimicrobial activity of spinosad against soil beneficial fungi

Soil Beneficial Fungi	Inhibition Zone diameters (mm)			Positive control
	Different concentration of spinosad			
	1*	5 *	10 *	Flucanozole (30 µg/ml)
Aspergillus fumigatus	12	13	14	16
Penicillium chrysogenum	6	6	6	17
Trichoderma viride	7	7	7	15
Mean ± S.D	8.33±3.21	8.66±3.78	9±4.35	16±1
CD (0.05)	7.98	9.40	10.83	2.48

*** Parts per Million**

Graph-2: Antimicrobial activity of spinosad against soil beneficial fungi



* Parts per Million

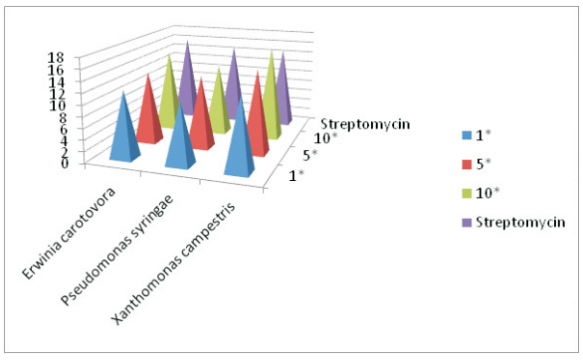
Antimicrobial activity of spinosad against plant pathogenic bacteria

The efficacy of spinosad was observed by performing antimicrobial activity of spinosad against plant pathogenic bacteria. The spinosad disc was prepared at different concentrations viz., 1 ppm, 5 ppm & 10 ppm and were tested against Erwinia carotovora, Pseudomonas syringae and Xanthomonas campestris Streptomycin was used as a positive control and the results were tabulated (Table-3) and graphical representation in (graph-3).The spinosad disc exhibited pronounced inhibition to all organisms. The maximum inhibition was 13 mm, 15 mm & 17 mm with respect to 1 ppm, 5 ppm & 10 ppm which was observed in Xanthomonas campestris. The zone of inhibition was 12 mm, 13 mm & 15 mm with respect to 1 ppm, 5 ppm & 10 ppm concentration in case of Erwinia carotovora and the inhibition of 11 mm, 11 mm & 13 mm at above said concentration (ppm) was observed in Pseudomonas syringae.

Table-3: Antimicrobial activity of spinosad against plant pathogenic bacteria

Plant Pathogenic Bacteria	Inhibition Zone diameters (mm)			Positive control
	Different concentration of spinosad			
	1 *	5 *	10 *	Streptomycin (30 µg/ml)
Erwinia carotovora	12	13	15	16
Pseudomonas syringae	11	13	13	15
Xanthomonas campestris	13	15	17	15
Mean ± S.D	12±7	13.66±1.15	15±2	15.33±0.5
CD (0.05)	2.48	2.86	4.96	1.43

Graph-3: Antimicrobial activity of spinosad against plant pathogenic bacteria



* Parts per Million

Antimicrobial activity of spinosad against plant pathogenic fungi

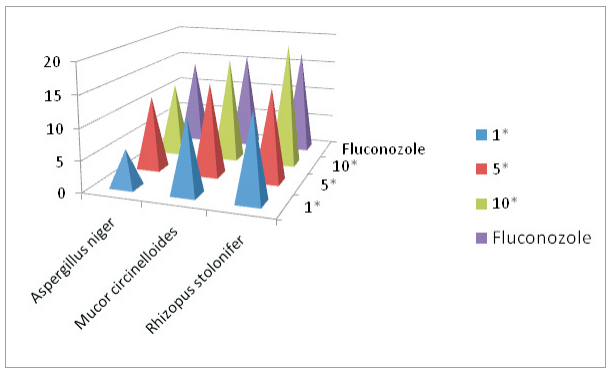
The efficacy of spinosad was observed by performing antimicrobial activity of spinosad against plant pathogenic fungi were tested. The spinosad disc was prepared at different concentrations viz., 1 ppm, 5 ppm & 10 ppm and antimicrobial activity was tested against Aspergillus niger, Mucor circinelloides and Rhizopus stolonifer. Flucanozole as a positive control was used in this test and the results were tabulated (Table-4) and graphical representation in (graph-4). The maximum inhibition of spinosad against Rhizopus stolonifer was observed, the zone of inhibition was recorded as 14 mm, 15 mm & 20 mm with respect to 1 ppm, 5 ppm & 10 ppm. The Mucor circinelloides with zone of inhibition of 12 mm, 15 mm & 17 mm with respect to 1 ppm, 5 ppm & 10 ppm. The minimal zone of inhibition was recorded as 6 mm, 12 mm & 12 mm with respect to 1 ppm, 5 ppm & 10 ppm in case of Aspergillus niger.

Table-4: Antimicrobial activity of spinosad against plant pathogenic fungi

Plant Pathogenic Fungi	Inhibition Zone diameters (mm)			Positive control
	Different concentration of spinosad			
	1 *	5 *	10 *	Flucanozole (30 µg/ml)
Aspergillus niger	6	12	12	14
Mucor circinelloides	12	15	17	16
Rhizopus stolonifer	14	15	20	17
Mean ± S.D	10.66±4.16	14±1.73	16.33±4.04	15.66±1.52
CD (0.05)	10.34	4.30	10.03	3.79

* Parts per Million

Graph-4: Antimicrobial activity of spinosad against plant pathogenic fungi



* Parts per Million

DISCUSSION:

The spinosad disc showed pronounced inhibition to plant pathogenic microbes which was tested such that bacteria viz., Erwinia carotovora, Pseudomonas syringae, and Xanthomonas campestris and also fungi viz., Aspergillus niger, Rhizopus stolonifer and Mucor circinelloides showed pronounced inhibition. Whereas in case of soil beneficial microbes exhibited minimal inhibition activity of spinosad. From this, we can interpret that spinosad act as ecofriendly so it does not affect the soil beneficial organisms. Apart from this, there is no other evidence or an article to show the relationship between spinosad and soil microorganisms. According to (Kukreja Girish P et al 2010), isolates of Pseudomonas showed activity against Xanthomonas and Aspergillus which is similar organism in our study among those organism the culture supernatant of Pseudomonas isolates V and X from graveyard soil showed antimicrobial activity against Xanthomonas (bacteria) and Aspergillus(fungi).

Beauvericin, a bioactive compound has strong antibacterial activity against plant pathogenic bacteria with no selectively between gram positive and gram negative bacteria (Qinggi wang and Lijian Xu, 2012). Beauvericin is

a biopesticide produced by fungi whereas spinosad, a microbial metabolite produced by an actinomycete, a soil dwelling bacterium also it has a strong activity against plant pathogenic bacteria as well as fungi.

Walsh et al., 2003 investigated with two natural products Eugenol and thymol (biostatic agent), against gram negative bacteria using MIC method, as we worked on the naturally derived spinosad using disc diffusion method. The aim is to find the activity of natural product against the organisms in both the cases is same but the method used is different.

Brkovic L.dusko et al., 2006 worked on 12 different plants from the family of Apiaceae. They screened the plants and tested against plant pathogenic bacteria such as Erwinia carotovora and Pseudomonas sp., as we used in our study. The other similarity is that the method used to find the antibacterial activity of 12 plant extracts (water, ethanol, ethyl acetate) against phytopathogenic bacteria by disc diffusion method. This study also showed that the most resistant bacteria was Erwinia carotovora to almost all the 12 plant extracts. Whereas in our study the Biopesticide spinosad have pronounced inhibition against the plant pathogen Erwinia carotovora.

Efficacy of fungicides and essential oils against phytopathogenic bacteria viz., Erwinia, Pseudomonas syringae, and Xanthomonas were tested. The target organism used in (Artur mikicinski et al., 2012) study was very similar to that of our study, but compound we tested was spinosad. The fungicides used in their study were Miedzian 50 WG (active substance – a.s. 50% copper oxychloride), Ridomil MZ Gold 68 WG (a.s. 3.8% metalaxyl-M and 64%, mancozeb), Euparen Multi 50 WG (a.s. 50% tolylfluanid), Captan 80 WG [a.s. 80% N-(captan)], Dithane Neotec 75 WG (a.s. 75% mancozeb). And the essential oils: such as lavender, sage, lemon balm, clove, and a preparation based on thyme oil (BioZell) were used to test the activity.Ridomil showed the strongest activity with largest inhibition zones for Xanthomonas which was a similar result in our study also. The fungicide captan showed no inhibition against Pseudomonas syringae, and Xanthomonas.whereas our Biopesticide showed strongest activity. Out of the essential oils lavender and lemon balm showed the lowest inhibition potential and no activity against Pseudomonas syringae.

Spinosad, a soil dwelling bacterium, (Biopesticide) which is harmless to soil beneficial microbes and has been tested against Azotobacter chroococcum, Pseudomonas fluorescens and Rhizobium leguminosarum and found that no zone of inhibition was observed in Azotobacter chroococcum and Pseudomonas fluorescens but minimum zone of inhibition was observed in Rhizobium leguminosarum which is not appreciable. The results of our research findings concluded that our test compound, a microbial metabolite has no harmful effect on soil beneficial organisms in contrast to plant pathogen, since the growth was inhibited by them as investigated.

CONCLUSION:

In the current study the activity of Spinosad against soil beneficial and plant pathogenic microbes was tested. The actinomycete metabolite Spinosad showed no activity against soil beneficial organisms whereas it showed significant activity against plant pathogenic microbes. The result of our study confirms that Spinosad can be used a natural bio pest control agent which has twin benefits.

REFERENCE:

I.Artur Mikiciński., Piotr Sobiczewski., Stanisław Berczyński. 2012 Efficacy of fungicides and essential oils against bacterial diseases of fruit trees. Journal of plant protection research vol. 52, no. 4.

II.Brković L. Duško., Ljiljana Comić., Slavica Solujić-Sukdolak. 2006. Antibacterial activity of some plants III.from family apiaceae in relation to selected phytopathogenic bacteria. kragujevac j. sci. 28:65-72.

IV.Cramer D.A., Barton R.A., Shorland F.B., Chochanska .Z. 1967. A comparison of the effects of white clover (Trifolium repens) and of perennial ryegrass (Lolium perenne) on fat composition and flavour of lamb. J. Agric. Sci., 69: 367-373.

V.Crouse G.D., Sparks T.C.1998. "Naturally derived materials as products and leads for insect control: The spinosyns",Rev. Toxicol., 2, 133—146.

VI.Kukreja Girish P., Bhute Shrikant S., Mangate Sunil A., Dhawale Manish N. 2010. Exploring the potential of Pseudomonas species as phosphate solubilizer, plant growth promoter, biocontrol agent and pesticide degrader.Original article. Asian j. exp. biol. sci.spl. 40-44.

VII.Liu T.U., Sparks A.N., Hendrix W.H., Yue B.1999. Effects of spintor on cabbage looper.toxicology and persistence of leaf residue on cabbage under field and laboratory conditions.J.econ.entomol.92:1266-1273.

VIII.Mertz E.P., Yao R.C. 1990. Saccharopolyspora spinosad sp nov. Isolated from soil collected in a sugar rum still. Int. J. Syst. Bacteriol., 40: 34–9.

IX.Naresh kumar .A., Murugan.K., Madhiyazhagan. P., Prabhu.K. 2011. Spinosad and Neem Seed Kernel Extract as Bio-Controlling agents for Malarial Vector, Anopheles stephensi and Non-Biting Midge,Chironomus circumdatus Asian Pacific Journal of Tropical Biomedicine 1-5.

X.Qinggui Wang., Lijian Xu. 2012. Beauvericin, a Bioactive Compound Produced by Fungi:A Short Review. Molecules, 17, 2367-2377.

XI.Thompson.G., Hutchins.S.1999 .Spinosad pesticide outlook,10:78-81.

XII.Walsh S.E., Maillard J.Y., Russell A.D., Catrenich C.E., Charbonneau D.L., Bartolo R.G. 2003. Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria , the Society for Applied Microbiology, Journal of Applied Microbiology, 94, 240–247.

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