



## RESEARCH ARTICLE

# Safety of spinetoram 12 SC to honey bees and earthworms

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## ABSTRACT

Investigations were carried out to assess the contact toxicity of spinetoram 12 SC to workers of Indian bee, *Apis cerana indica* and earthworm species *Eudrilus eugeniae*. Contact toxicity of spinetoram 12 SC to Indian bees, *A. cerana indica* F. showed that there was no mortality recorded due to spinetoram 12 SC at 36 g a.i./ha (very low dose of biological insecticide spinetoram 12 SC), while spinetoram 12 SC at 45 g a.i./ha (2.6%) and spinetoram 12 SC at 54 g a.i./ha (8.3%) at 6 HAT were least toxic. Emamectin benzoate 5 SG at 8.5 g a.i./ha (10.4%), spinetoram 12 SC at 108 g a.i./ha (21.5%) were slightly toxic. The mortality greater than before as the time of exposure increased from 6 to 24 HAT. Toxicity of spinetoram 12 SC to earthworm, *E. eugeniae* showed that there was lowest mortality recorded in spinetoram 12 SC at 36 g a.i./ha (1.2%) followed by spinetoram 12 SC at 45 g a.i./ha (5.3%) and spinetoram 12 SC at 54 g a.i./ha (13.7%) at 7 DAT. Emamectin benzoate 5 SG at 8.5 g a.i./ha (14.3%), spinetoram 12 SC at 108 g a.i./ha (19.7%) were moderately toxic. At 7 DAT, percent mortality was recorded in Novaluron 10 EC at 75 g a.i./ha (21.7%), quinalphos 25 EC at 200 g a.i./ha (26.3%), chlorpyrifos 20 EC at 200 g a.i./ha (27.0%), cypermethrin 25 EC at 50 g a.i./ha (37.3%) and indoxacarb 14.5 SC at 75 g a.i./ha (39.0%). The mortality increased as the time of exposure increased from 7 to 14 DAT. Hence, spinetoram 12 SC considered could be highly safe to Indian honey bee species *Apis cerana indica* and earthworm species *Eudrilus eugeniae* than standard insecticides

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## INTRODUCTION

Because of the haphazard use of pesticides with various mode of action, bring the danger of resistance development, pest resurgence, outbreaks of secondary pests, reduction in biodiversity of natural enemies and bio-concentrations of residues in consumable produces at harvest have opened the new era of eco-friendly insecticides having novel mode of action with higher activity against target insects. One such insecticide is spinetoram 12 SC w/v (11.7 w/w) that belongs to spinosyn group. This new group showed increased efficacy against many caterpillars and thrips and relatively safe to non-target organisms like parasitoids, predators, honeybees and earthworms.

Bees and other pollinators not only provide services to the ecosystem but also to humans (Jadeja, 2010). Honeybees are considered as the most efficient and reliable pollinators of varied crops (McGeor, 1976 and Klein *et al.*, 2007). In most of the crops we mostly seek honey bees and depend on them for pollination services (Goswami *et al.*,

2013). As pollinators, honeybees played an essential role in increasing the yield of vegetables. However, recent declines in pollination populations have affected global agricultural production and affect both food production and the economy (Potts *et al.*, 2010). These pollinating bees are exposed directly to insecticides during spraying, as well as to the left over insecticides on the crop.

In the total biomass of terrestrial invertebrates, a larger proportion is 80% represented by earthworms, which play a vital role in structuring and escalating the nutrient content of the soil. Therefore, they can be appropriate bioindicators of chemical pollution of the soil in global bionetwork providing an early caution of worsening in soil quality (Culy & Berry, 1995; Sorour & Larink, 2001 and Bustos – Obregon & Goicochea, 2002). This is important for protecting the health of natural environments and is of increasing interest in the context of protecting human health (Beeby, 2001) as well as other terrestrial vertebrates that prey upon earthworms (Dellomo *et al.*, 1999). The suitability of earthworms as bioindicators in soil

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toxicity is largely due to the fact that they ingest a large quantity of the decomposed litter, manure, and other organic matter deposited on soil, helping to convert it into rich topsoil (Reinecke & Reinecke, 1999 and Sandoval *et al.*, 2001). Moreover, studies have shown that earthworm skin is a significant route of contaminant uptake (Lord *et al.*, 1980).

Understanding the toxicity of insecticides to these beneficial organisms is important and relevant to develop a sound pest management program (Neetan & Naveen Aggarwal, 2012). To find out the toxicity and safety levels of spinetoram and other insecticides on the above beneficial organisms are essential to integrate this spinetoram 12 SC to safer pest management practices. So, this investigation was undertaken with the objectives to study the toxicity and safety level of spinosyn, spinetoram 12 SC against honeybee species, *Apis cerana indica* F. and earthworm, *Eudrilus eugeniae* (Kinberg).

## MATERIAL AND METHODS

### Toxicity of spinetoram 12 SC to honey bees:

Indian bee, *Apis cerana indica* (Fab.) hive with queen, drones and workers was obtained from the Ms. Vibis Honey Bee Training Centre, Alanganallur and maintained in the Insectary of Agricultural College and Research Institute, Madurai. Workers from the hives were used for toxicity studies.

A laboratory experiment was conducted to assess the toxicity of spinetoram 12 SC to workers of Indian bee, *Apis cerana indica*. Earlier, honeybees were kept in the refrigerator prior to the test to make them calm and for easier transfer. The experiment consisted of treatments (0.6, 0.75, 0.9 and 1.8 ml of spinetoram 12 SC; 0.34 g of emamectin benzoate 5 SG; 0.4 ml of cypermethrin 25 EC; 2 ml of chlorpyrifos 20 EC; 1.6 ml of quinalphos 25 EC; 1.04 ml of indoxacarb 14.5 SC; and 1.5 ml of novaluron 10 EC and untreated control), which were fixed based on field doses, and each treatment was replicated three times. The effect of spinetoram 12 SC to honey bees was assessed by contact toxicity method. Various concentrations of spinetoram 12 SC were prepared using distilled water. For giving adequate aeration to the bees, plastic containers that have perforations in the lid were used for this study. Then filter papers were placed inside the container. After that the filter papers were wetted with one ml of different concentrations of the chemicals and then allowed to dry. Then 10 numbers of honeybees were transferred in each container. After exposure to one hour, a 40 per cent sucrose solution soaked in cotton wool was given as feed for the honeybees (Suganyakanna, 2006). Then the mortality of honeybee was observed after 6, 12 and 24 h of treatment and per cent mortality worked out.

### Toxicity of spinetoram 12 SC to earth worms *E. eugeniae*:

The nucleus culture of *Eudrilus eugeniae* (Kinberg) was obtained from the Department of Soil Science and Environment, AC&RI, Madurai and maintained on earthen soil pots in the Insectary, and used for toxicity studies.

The effect of spinetoram 12 SC on earthworm *E. eugeniae* was tested by following the artificial soil test method proposed by Biologische Bundesanstalt für Land-und Forstwirtschaft, Braunschweig (BBA) as accounted by Ganeshkumar (2000). One kg of conditioned soil in tubular pots (18 x 6 cm) was treated with insecticides mentioned in the toxicity of spinetoram 12 SC to honey bees' methodology. A total number of 15 earthworms were washed cleanly in water and placed on the top of the substrate. The tubular pots were covered with perforated polythene cover to prevent the worms from crawling out and to avoid evaporation. The set up was kept under shade. The number of dead and live earthworms was counted after 7 and 14 DAT and percent mortality were calculated. Earthworms were considered dead if they did not respond to a gentle mechanical stimulus (Edwards and Bohlen, 1992). An untreated control was maintained throughout the experiment.

### Statistical analysis:

The data from various laboratory experiments were scrutinized by CRD analysis of variance (ANOVA) after getting transformed into  $x+0.5$  and arcsine percentage values where appropriate (Gomez and Gomez, 1984). The per cent mortality in laboratory studies was corrected using Abbot's formula (Abbot, 1925),

$$\text{Per cent corrected mortality} = \frac{\text{Per cent test mortality} - \text{per cent control mortality}}{100 - \text{per cent control mortality}} \times 100$$

## RESULTS AND DISCUSSION

### Evaluation of toxicity of spinetoram 12 SC to honeybee, *Apis cerana indica* F.

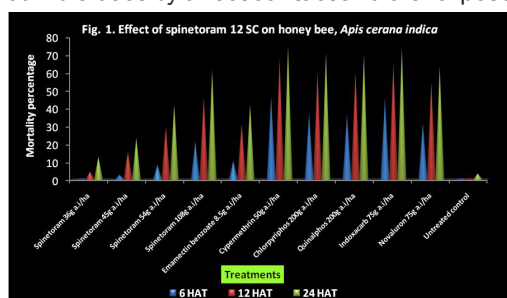
Contact toxicity of spinetoram 12 SC to Indian bees, *A. cerana indica* F. showed that there was no mortality recorded due to spinetoram 12 SC at 36 g a.i./ha (0.0%), while spinetoram 12 SC at 45 g a.i./ha (2.6%) and spinetoram 12 SC at 54 g a.i./ha (8.3%) at 6 HAT were least toxic. Emamectin benzoate 5 SG at 8.5 g a.i./ha (10.4%), spinetoram 12 SC at 108 g a.i./ha (21.5%) were slightly toxic. However, cypermethrin 25 EC at 50 g a.i./ha, quinalphos 25 EC at 200 g a.i./ha, chlorpyrifos 20 EC at 200 g a.i./ha, indoxacarb 14.5 SC at 75 g a.i./ha and novaluron 10 EC at 75 g a.i./ha registered more than 30.00 per cent mortality at 6 HAT.

At 12 HAT, spinetoram 12 SC at 36 and 45 g a.i./ha were on par and registered 4.3 and 15.4 per

cent mortality on honeybees. This was followed by spinetoram 12 SC 54 g a.i./ha (29.2%). Spinetoram 12 SC 108 g a.i./ha however, registered mortality of 45.8 per cent which was followed by novaluron 10 EC at 75 g a.i./ha (54.4%). Cypermethrin 25 EC at 50 g a.i./ha, quinalphos 25 EC at 200 g a.i./ha, chlorpyrifos 20 EC at 200 g a.i./ha and indoxacarb 14.5 SC at 75 g a.i./ha recorded more than 60.0 per cent mortality of Indian bees at 12 HAT.

At 24 HAT, exposure to the lower dose of spinetoram 12 SC at 36 g a.i./ha resulted in per cent mortality of 12.7 which was followed by spinetoram 12 SC at 45 g a.i./ha (23.4%). Spinetoram 12 SC 54 g a.i./ha (42.2%) and emamectin benzoate 5 SG at 8.5 g a.i./ha (42.3%) were equally effective and were followed by spinetoram 12 SC at 108 g a.i./ha (61.8%). All the other insecticidal treatments recorded more than 70.0 per cent mortality of *A. cerana indica*. The mortality increased as the time of exposure increased from 6 to 24 HAT (Fig. 1).

The results were in agreement with Besard *et al.* (2011) who reported that spinetoram 12 SC is safer for bumble bees by direct contact and oral exposure



than the use of spinosad, and therefore spinetoram can be safer for bees. Direct contact of *Bombus terrestris* L. workers with wet residues of spinosad and spinetoram showed spinetoram 12 SC to be approximately 52 times less toxic than spinosad, while oral treatment for 72 hr (acute) indicated that spinetoram 12 SC was about 4 times less toxic to *B. terrestris* compared with spinosad. During exposure of bumblebees to spinosad there was no effect on bumblebee colony health, adult bee mortality, brood development, emergence of young bees and foraging efficiency of adults that underwent larval development (Morandin *et al.*, 2005). According to Miles and Dutton (2000), spinosad was safe to foraging bees. But Cleveland *et al.* (2002) and Miles (2003) stated that spinosad was acutely toxic to bees under lab conditions, but field studies indicated that under actual conditions the impact on bees was minimal.

Cypermethrin recorded the highest per cent mortality after a day of treatment that falls in line with Perry (1998) who reported that the synthetic pyrethroids and conventional insecticides were

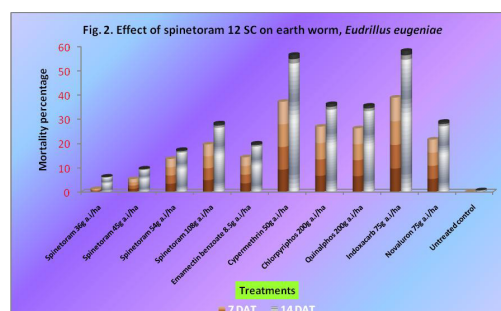
considered to be highly or extremely toxic to honeybees based on laboratory acute toxicity tests. Since most of the vegetables are often cross-pollinated crops and rich in pollen, spinetoram 12 SC will be useful in managing fruit borers effectively with minimal hazards to honeybees.

### Evaluation of toxicity of spinetoram 12 SC to earthworm, *Eudrilus eugeniae* (Kinberg):

Toxicity of spinetoram 12 SC to earthworm, *E. eugeniae* showed that there was lowest mortality recorded in spinetoram 12 SC at 36 g a.i./ha (1.2%) followed by spinetoram 12 SC at 45 g a.i./ha (5.3%) and spinetoram 12 SC at 54 g a.i./ha (13.7%) at 7 DAT. Emamectin benzoate 5 SG at 8.5 g a.i./ha (14.3%), spinetoram 12 SC at 108 g a.i./ha (19.7%) were moderately toxic. Novaluron 10 EC at 75 g a.i./ha, quinalphos 25 EC at 200 g a.i./ha, chlorpyrifos 20 EC at 200 g a.i./ha, cypermethrin 25 EC at 50 g a.i./ha and indoxacarb 14.5 SC at 75 g a.i./ha however registered 21.7, 26.3, 27.0, 37.3 and 39.0 per cent mortality at 7 DAT.

At 14 DAT, exposure to the lower doses of spinetoram 12 SC at 36 and 45 g a.i./ha was on par and registered percent mortality of 6.3 and 9.7. This was followed by spinetoram 12 SC at 54 g a.i./ha (17.4%). Emamectin benzoate 5 SG at 8.5 g a.i./ha (20.0%) and spinetoram 12 SC at 108 g a.i./ha (28.3%) were moderately toxic. At 14 DAT, percent mortality of earthworms in different insecticides were recorded as novaluron 10 EC at 75 g a.i./ha (29.0%), quinalphos 25 EC at 200 g a.i./ha (35.7%), chlorpyrifos 20 EC at 200 g a.i./ha (36.3%), cypermethrin 25 EC at 50 g a.i./ha (56.7%) and indoxacarb 14.5 SC at 75 g a.i./ha (58.3%). The mortality greater than before as the time of exposure increased from 7 to 14 DAT (Fig 2).

These findings are in agreement with the report of Anonymous (2007) who indicated that earthworms were exposed to spinetoram 12 SC treated soil and



evaluated for acute toxicity after 14 days of exposure in which LC50 was > 1000 ppm. According to Robidoux *et al.* (1999) who affirmed that mortality per cent has been the most commonly used parameter to evaluate the chemical toxicity in earthworms. Some studies indicated that juveniles



are often more sensitive to chemicals than adults (Booth and O'Halloran, 2001). Alshawish (2004) reported that most toxicity tests for cypermethrin on earthworms were conducted using adult earthworms. Earthworms were exposed to sub-lethal and lethal concentrations of chlorpyrifos and cypermethrin and evaluated for acute toxicity, growth, fecundity and avoidance response after certain exposure period in which 78.91 mg/kg chlorpyrifos and 10 mg/kg cypermethrin caused significant toxic effects in all test methods (Shi-ping et al., 2006, 2008). Spinetoram 12 SC granular formulation did not cause any detrimental effects on earthworms and aquatic organisms (Anonymous, 2008).

## CONCLUSION

Extensive use of broad-spectrum synthetic chemicals results in the destruction of non-target organisms when they are transported. These synthetic insecticides have a long life period, which has resulted in the process of bioaccumulation and biomagnification in the environment and in living organisms. So understanding the toxicity of insecticides to these beneficial organisms is important and relevant to develop a safe and sound pest management program. Hence, based on the above research results showed that spinetoram 12 SC was found comparatively less toxic to Indian honeybee species *Apis cerana indica* and earthworm species *Eudrilus eugeniae* than standard insecticides.

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## REFERENCES

- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18**: 265-267.
- Alshawish, S.A., Mohamed, A. I. and G. A. Nair. 2004. Prolonged toxicity of sublethal dosages of chemical pesticides on the body mass and cocoons of *Aporrectodea caliginosa* (Savigny) (Oligochaeta: Lumbricidae) inhabiting Benghazi, Libya. *Proc. Nat. Acad. Sci. India*. **74**(2): 123-133.
- Anonymous. 2007. California Department of Pesticide Regulation. Public Report 2007–2010: Spinetoram. 1-10.
- Anonymous. 2008. Dow Crop Science Spinetoram. *Technical Bulletin* 1-6.
- Beeby, A. "What do sentinels stand for?" *Environmental Pollution*, vol. 112, no. 2, 285–298, 2001.
- Besard, L., Mommaerts, V., Abdu-alla, G. and G. Smagghe. 2011. Lethal and sublethal side effect assessment supports a more benign profile of spinetoram compared with spinosad in the bumblebee *Bombus terrestris*. *Pest Manag. Sci.* **67**(5): 541-547.
- Booth, L.H. and K.O'Halloran. 2001. A comparison of biomarker responses in the earthworm *Aporrectodea caliginosa* to the organophosphorus insecticides diazinon and chlorpyrifos. *Ecotoxicol. Environ. Saf.*, **20**: 2494-2502.
- Bustos -Obregón, E. and R.I. Goicochea. 2002. "Pesticide soil contamination mainly affects earthworm male reproductive parameters," *Asian J. Andrology*, **4**(3): 195–199.
- Cleveland, C. B., Mayer, M. A. and S. A. Cryer. 2002. An ecological risk assessment for spinosad use on cotton. *Pest Mgt. Sci.*, **58**(1): 70-84.
- Culy, M. D. and E. C. Berry. 1995. "Toxicity of soil-applied granular insecticides to earthworm populations in cornfields," *Down to Earth*, **50**: 20–25.
- Dell'Omo, G., Turk, A. and R. F. Shore. 1999. "Secondary poisoning in the common shrew (*Sorex araneus*) fed earthworms exposed to an organophosphate pesticide," *Environ. Toxicol. Chemistry*, **18**(2): 237–240.
- Edwards, C. D. and P.J. Bohlen. 1992. The effects of toxic chemicals on earthworms. *Rev. Environ. Contam. Toxicol.*, **125**: 24-99.
- Ganeshkumar. M. 2000. Effect of pesticides on earthworm. In: *Proc. of summer school on environmental impact of pesticides in Agro eco system*. May 2-23, 2000. G. Santharam, R. Jayakumar, S. Kuttalam, S. Chandrasekaran and T. Manoharan (eds.), TNAU. Coimbatore. 57-61.
- Gomez, K.A. and A.A. Gomez. 1984. *Statistical procedures for Agricultural Research*. A Wiley International Science Publication, John Wiley and Sons, New Delhi. 680.
- Goswami, V., Khan, M. S. and Usha. 2013. Studies on pollinator fauna and their relative abundance of sunflower at Pantnagar, Uttarkhand. *Indian J. Appl. Nature Sci.*, **5**: 294.
- Jadeja, S. Ecosystem Services. *Science Reporter*. **47**: 45.
- Klein, A.M., Vaissiere, B.E., Cane, J.H., Steffan - Dewenter, I., Cunningham, S.A., Kremen, C. and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. *Proc. Biol. Sci.*, **274**: 303.
- Lord, K. A., Briggs, G.G., Neale, M. C. and R. Manlove. 1980. "Uptake of pesticides from water and soil by earthworms," *Pesticide Sci.*, **11**(4): 401–408.
- Mc Greor, S.E. 1976. Insect pollinators of cultivated crop plants. U.S.D.A. Publication, USA, 411.
- Miles, M. 2003. The effect of spinosad a naturally derived insect control agent to the honey bee. *Bull. Insectology*. **56**(1): 119-124.
- Miles, M. and R. Dutton, 2000. Spinosad: a naturally derived insect control agent with potential use in glasshouse integrated pest management systems.

- Medede. Fac. Landbouwkundige Toegepaste Biol. Wet.University Gent, **65**: 393–400
- Morandin, L. A., Winston, M. L., Franklin, M. T. and V. A. Abbott. 2005. Lethal and sub lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson). *Pest Manag. Sci.*, **2(1)**: 23-30.
- Neetan and N. Aggarwal. 2012. Toxicity of some insect growth regulator and neonicotinoid insecticides to cotton mealybug predator, *Cheilomenes sexmaculata*. *Madras Agric. J.*, **99(1-3)**: 116-120.
- Perry, J. N. 1998. Measures of spatial pattern and spatial association for counts of insects. –In: Baumgartner, J., Brandmayr, P. and Manly, B.F. J. (eds), *Population and community ecology for insect management and conservation*. Balkema, pp. 21–33.
- Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. *Trens Ecol. Evol.*, **25**: 345.
- Reinecke, S. A. and A. J. Reinecke. 1999. "Lysosomal response of earthworm coelomocytes induced by longterm experimental exposure to heavy metals," *Pedobiologia*, **43(6)**: 585–593.
- Robidoux, P.Y., Hawari, J., Thiboutot, S., Ampleman, G. and G.I. Sunahara. 1999. Acute toxicity of 2, 4, 6 - trinitrotoluene in earthworm (*Eisenia Andrei*). *Ecotoxicology and Environmental Safety*, **44(3)**: 311-321.
- Sandoval, M. C., Veiga, M., Hinton, J. and B. Klein. 2001. "Review of biological indicators for metal mining effluents: a proposed protocol using earthworms," in *Proceedings of the 25<sup>th</sup> Annual British Columbia Reclamation Symposium*, 67–79.
- Shi-ping, Z., Chang-qun, D., Hui, C., Yu-hui, F., Xue-hua, W. and Y. Ze-fen. 2006. Toxicity assessment for chlorpyrifos - contaminated soil with three different earthworm test methods. *J. Environ. Sci.*, **19**: 854-858.
- Shi-ping, Z., Chang-qun, D., Hui, C., Yu-hui, F., Xue-hua, W. and Y. Ze-fen. 2008. Assessing cypermethrin-contaminated soil with three different earthworm test methods. *J. Environ. Sci.*, **20**: 1381-1385.
- Sorour, J. and O. Larink. 2001. "Toxic effects of benomyl on the ultrastructure during spermatogenesis of the earthworm *Eisenia fetida*." *Ecotoxicology and Environmental Safety*. **50 (3)**: 180–188.
- Suganyakanna, S. 2006. Evaluation of acetamiprid 20 SP against sucking pest complex in cotton. Ph.D. Thesis. Tamil Nadu Agric. Univ., Coimbatore-3, India, 190.