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# Could biorational insecticides be used in the management of aflatoxigenic *Aspergillus* spp. and their insect vectors in stored wheat?

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Insect pests in stored wheat cause significant losses and play an important role in the dispersal of viable fungal spores of various species including aflatoxin producing *Aspergillus* spp. The problems of insecticide resistance in stored insects and environmental hazards associated with fumigants and conventional grain protectants underscore the need to explore reduced risk insecticides to control stored insects and the ultimate effect on fungal infection inhibition. The purpose of this study was to investigate the insecticidal potential of four biorational insecticides: spinosad, thiamethoxam, imidacloprid and indoxacarb on wheat against *Rhyzopertha dominica* and *Sitophilus oryzae* and the subsequent effect of insects' mortality on *Aspergillus flavus* and *A. parasiticus* infection in grains. Spinosad and thiamethoxam were the most effective insecticides against *R. dominica* compared to *S. oryzae* followed by imidacloprid. Spinosad applied at 0.25, 0.5 and 1ppm and thiamethoxam at 2 and 4ppm concentrations resulted in complete mortality of *R. dominica* and >90% infection inhibition of *A. flavus* and *A. parasiticus*. However, indoxacarb was more toxic against *S. oryzae* compared to *R. dominica*. The mortality of *R. dominica* was directly related to the percent infection inhibition of *A. flavus* and *A. parasiticus* in all the treatments. Whereas, mortality of *S. oryzae* was only related to the percent infection inhibition of *A. parasiticus* in all the treatments. The results show that although both spinosad and thiamethoxam can provide protection against *R. dominica* and fungal infections in stored grains, more potent reduced risk insecticides and/or their combinations would be needed than either of these to provide broad spectrum protection of stored grains. In conclusion, the results of the present study provide baseline data for the management of aflatoxigenic fungi by controlling stored insects using biorational insecticides .

**Could biorational insecticides be used in the management of aflatoxigenic *Aspergillus* spp.  
and their insect vectors in stored wheat?**

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27 *parasiticus* infection in grains. Spinosad and thiamethoxam were the most effective insecticides  
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## Introduction

Aflatoxins are the group of structurally diverse mycotoxins that are mainly produced by *Aspergillus flavus* and *A. parasiticus*, both belonging to section *Flavi* [1,2]. These mycotoxins are recognized as immunosuppressive, carcinogenic, hepatotoxic, mutagenic and teratogenic [3], since they lead to serious human and animal health hazards, including acute and chronic liver diseases, tumor induction, reproductive disorders, genotoxicity and nephrotoxicity [4-6]. These mycotoxins are known to contaminate more than 25% of the world stored grain cereal commodities of which more than 300 fungal metabolites are reported to cause human and animal toxicity [7].

Stored grain contamination with insect pests and fungi is a serious problem resulting in more than 20% losses in overall production by decreasing seed germination and downgrading of grains [8]. Contamination of stored grains with fungal spores is mainly a source of mycotoxins which usually results from stored insect pest's infestation [9]. Insects disseminate the fungal spores all over the grain bulk by their constant movement, which are carried on their body and/or deposited in insect frass [10]. These insects break seed coat as a natural barrier to fungus and provide entry point for fungal infection [11]. Therefore strategies to control insects are needed. The most serious insect pests of stored grains that cause >20% postharvest losses in developing countries are: rice weevil (*Sitophilus oryzae*), red flour beetle (*Tribolium castaneum*), lesser grain borer (*Rhyzopertha dominica*), rusty grain beetle (*Cryptolestes ferrugineus*) and khapra beetle (*Trogoderma granarium*) [8,12].

Control of these insects is of prime importance as they have the ability to carry and transmit spores of *Aspergillus* spp. internally [13]. Current control measures for these insects rely on the

extensive use of fumigants and conventional insecticides that have resulted in increased insect resistance [14], primary pest resurgence [15], secondary pest outbreak [16], and their use as grain protectant is being reconsidered for their effect on health and environmental safety [17]. Therefore, there is a need to explore reduced risk or biorational insecticides with minimal environmental effect and mammalian toxicity for judicious insect pest management.

Biorational insecticides are usually target specific new insecticides with low mammalian toxicity. Some of the biorational insecticides like spinosad, thiamethoxam have been proved as potential grain protectants against stored insect pests in different parts of the world [17, 18]. However, such studies are rare at Pakistan level. Therefore, in the present study, our aims were to evaluate the toxicity of spinosad, thiamethoxam, imidacloprid and indoxacarb against two major pests of stored wheat *R. dominica* and *S. oryzae*, and the subsequent effect of insects' mortality on *A. flavus* and *A. parasiticus* infection in wheat grains. The results would be helpful in the management of insects and aflatoxigenic fungi in stored commodities.

## Materials and Methods

### Commodities, formulations and fungal cultures

Untreated, clean and infestation free wheat grains (var Seher-06) with 11.1% moisture contents were used in the present study. The insecticide formulations were spinosad (Tracer® 24% active ingredients [IA], Arysta Life Sciences, Pakistan), thiamethoxam (Actara® 25% [AI], Syngenta, Pakistan), imidacloprid (Confidor® 20% [AI], Bayer Crop Sciences, Pakistan) and indoxacarb (Steward 15%, DuPont, Pakistan). *Aspergillus flavus* and *A. parasiticus* cultures were obtained from First Fungus Culture Bank, Pakistan (FFCB).

### Insects

Healthy cultures of *Rhyzopertha dominica* and *S. oryzae* adults were collected from grain market, Lahore (31.5497° N, 74.3436° E), and reared on whole wheat in the laboratory at 26±1 °C and 70±5%. The insects were reared for five generations before starting experiments. Adults of both species used in bioassays were mixed sex and 2-3-weeks-old [19].

### Bioassays

Insecticidal bioassays were done by following the methodology of Athanassiou et al. [19] with some modifications. Briefly, insecticides were applied as a solution diluted with distilled water. Individual replicate lots of 200 g of wheat grains were placed in 0.5 litre glass jars for insecticide treatments: 0 (control; the grains treated with distilled water), 0.25, 0.5 and 1 mg/kg for spinosad; 0 (control; the grains treated with distilled water), 1, 2 and 4 mg/kg for thiamethoxam; 0 (control; the grains treated with distilled water), 1, 2 and 4 mg/kg for imidacloprid; 0 (control; the grains treated with distilled water), 1, 2 and 4 mg/kg for indoxacarb. To achieve above concentrations, 2 mL solution of each treatment was prepared and applied to the 200 g grains. In order to maximize insecticide distribution, the jar was shaken manually for 5 minutes [19,21].

The jars were left in the laboratory for 24 h at 25 °C and complete darkness to dry. After 24 h, the jars with insecticide treated wheat grains were inoculated with 0.5 ml spore suspension ( $10^4$  spores/ml) of *A. flavus* (Arena “A”) or *A. parasiticus* (Arena “B”) separately. Twenty adults of *R. dominica* or *S. oryzae* were introduced in each jar, and the insects were disinfected with 0.5% sodium hypochlorite for 2 min and washed with sterile distilled water before entering into the jars [20]. To check the direct effect of insecticides on fungal infection inhibition, the same procedure was repeated but without introducing insects in the treated jars. The treated jars were closed with muslin cloth and maintained in the laboratory at  $26 \pm 1$  °C and  $70 \pm 5\%$ . After 14 days, the jars were opened for mortality counts and percent infection inhibition of *A. flavus* or *A. parasiticus*.

Percent infection inhibition was determined by following the methodology of Krishnamurthy et al. [21]. Briefly, 60 treated and insect infested wheat grains were withdrawn from the individual replicate and placed on three layers of sterilized moistened blotter discs in sterilized petri plates (90 mm diameter) at the rate of 20 grains per layer. The plates were incubated for 7 days in the laboratory at  $26 \pm 1$  °C. After incubation, the plates were examined under the stereobinocular microscope for the presence *A. flavus* or *A. parasiticus* and the number of fungal colonies and healthy seeds (i.e., without colony) were counted. Infection inhibition percentage was calculated as follows:

Percent infection inhibition = (number of healthy (uninfected) seeds/total number of seeds) \* 100

The entire procedure was replicated five times by preparing new lots of treated wheat for each replicate, and exposing, counting, and recording data for adult mortality and fungal infection as described above.

## Data analysis



122 Percent mortality and infection inhibition percentage data were analyzed separately for each  
123 insect and fungal species using a one-way analysis of variance (ANOVA) with Statistix 8.1  
124 software [22]. Means were compared by Tukey's Honestly Significant Difference (HSD) test, at  
125 0.05 probability.

126

## Results

### Mortality

In case of spinosad, mortality for *R. dominica* was higher than for *S. oryzae*. *Rhyzopertha dominica* showed 100% mortality at the spinosad concentrations of 0.25-1 mg/kg, in both the arena (F=6273; df=3, 16; p<0.01, for arena A, and F=2401; df=3, 16; p<0.01, for arena B). Whereas, *S. oryzae* showed the highest mortality at the concentration 1ppm followed by 0.5 and 0.25ppm (F=235; df=3, 16; p<0.01, for arena A, and F=218; df=3, 16; p<0.01, for arena B) (Fig. 1.).

*Rhyzopertha dominica* showed complete mortality on wheat treated with thiamethoxam at concentrations 2 and 4ppm (F=858; df=3, 16; p<0.01, for arena A, and F=262; df=3, 16; p<0.01, for arena B). Whereas, *S. oryzae* showed complete mortality only at 4 ppm, in both arena (F=1421; df=3, 16; p<0.01, for arena A, and F=498; df=3, 16; p<0.01, for arena B) (Fig. 2).

In case of imidacloprid, mortality for *R. dominica* was higher than for *S. oryzae*. *R. dominica* showed 92 and 94% mortality in arena A and B respectively, at the highest concentration 4ppm (F=234; df=3, 16; p<0.01, arena A, and F=192; df=3, 16; p<0.01, for arena B) (Fig. 3). Whereas, *S. oryzae* showed 33 and 39% mortality in arena A and B respectively, at the highest concentration 4ppm (F=43.6; df=3, 16; p<0.01, for arena A, and F=50.8; df=3, 16; p<0.01, for arena B).

For indoxacarb, in contrast, mortality for *R. dominica* was lower than for *S. oryzae*. *R. dominica* showed 94 and 92.3% mortality in arena A and B, respectively at the concentration 4ppm (F=140; df=3, 16; p<0.01, for arena A, and F=150; df=3, 16; p<0.01, for arena B). However, *S.*

148 *oryzae* showed complete mortality at 2 and 4ppm ( $F=403$ ;  $df=3, 16$ ;  $p<0.01$ , for arena A, and  
149  $F=661$ ;  $df=3, 16$ ;  $p<0.01$ , for arena B)

## 150 **Fungal infection inhibition**

151 Mortality of *R. dominica* seems to influence directly the percent infection inhibition of *A. flavus*  
152 and *A. parasiticus* in all the treatments. In the controls, where the survival percentage was high,  
153 less infection inhibition of both fungi was observed. In case of spinosad, where mortality of *R.*  
154 *dominica* was 100% at the tested concentrations, more than 93% infection inhibition of *A. flavus*  
155 and *A. parasiticus* was observed. Whereas, in controls where the survival percentage of *R.*  
156 *dominica* was high ( $>90\%$ ), significantly more incidence of both fungi was observed. The same  
157 trend was observed with rest of the insecticides tested (Fig. 1-4). Similarly, percent mortality of  
158 *S. oryzae* seems to influence only the infection inhibition of *A. parasiticus* (Fig. 1-4). Moreover,  
159 highly significant positive correlations ( $p<0.01$ ) were observed between mortality of *R. dominica*  
160 and infection inhibition of *A. flavus* or *A. parasiticus*, and mortality of *S. oryzae* and infection  
161 inhibition of *A. parasiticus* ( $p<0.01$ ; Table 1). However, in the present study there was  
162 statistically no effect of insecticides on fungal infection inhibition when the insecticides were  
163 tested without introducing insects in the treated jars (data not shown here).

164

## Discussion

The results of the present study revealed varying level of toxicities of insecticide against *R. dominica* and *S. oryzae*. The results also suggest that control of these insects may help limit the infection of aflatoxigenic fungi in stored wheat.

Overall, spinosad was the most effective insecticide against *R. dominica* compared to *S. oryzae*. Spinosad applied at 0.25, 0.5 and 1ppm concentrations resulted in complete mortality of *R. dominica*. In contrast, *S. oryzae* showed 94% mortality at the highest concentration of spinosad tested. Spinosad is a relatively new insecticide of low mammalian toxicity and has a broad spectrum of target species, including stored insect pests [23], and has been registered for use in the United States [19]. The findings on spinosad toxicity against the tested insect species are in agreement with a number of previous reports [19,23-26]. It seems that if *R. dominica* is the sole species in stored commodities, spinosad may be as an effective grain protectant.

In case of thiamethoxam, *R. dominica* showed complete mortality at concentrations 2 and 4ppm, whereas *S. oryzae* showed complete mortality at only 4 ppm. Thiamethoxam is a neonicotinoid and a relatively new insecticide of low mammalian toxicity and does not produce mutagenic and/or teratogenic effects [18]. It has been extensively used as a seed coat treatment with satisfactory results against a wide range of pests [27]. Thiamethoxam was the first time evaluated as a grain protectant by Arthur et al. [18] and found it effective against *R. dominica* and *S. oryzae*. It was further reported that the mortality of *S. oryzae* and *R. dominica* increased with time and it had reached approximately 100% after 6 d of exposure to thiamethoxam. But in our study, *S. oryzae*, showed less than 100% mortality at 2ppm after 14 d of exposure. This difference could be due to the different origin of *S. oryzae*.

187 Imidacloprid is also a neonicotinoid with low mammalian toxicity and has been used as seed  
 188 treatment for the management of a number of sucking insect pests [27]. Recently, Daglish et al.  
 189 [28] investigated the potential of imidacloprid against different beetle pests of stored grains.  
 190 They reported that the toxicity of imidacloprid was species and dose dependent, and that *R.*  
 191 *dominica* was more susceptible to imidacloprid than *S. oryzae*. The results of the present study  
 192 with respect to imidacloprid also revealed that the mortality for *R. dominica* was higher than for  
 193 *S. oryzae*. *Rhyzopertha dominica* showed up to 94% mortality at the highest concentration 4 ppm  
 194 as compared to 39% mortality for *S. oryzae*.

195 Indoxacarb belongs to an oxadiazine insecticide group and has strong potential against different  
 196 insect pests [29]. In the present study mortality for *R. dominica* was lower than for *S. oryzae*,  
 197 which is in agreement with Daglish et al. [28] who evaluated indoxacarb potential as a grain  
 198 protectant first time.

199 Concerning the infection inhibition of tested fungi, mortality of *R. dominica* was directly related  
 200 to the percent infection inhibition of *A. flavus* and *A. parasiticus* in all the treatments. In the  
 201 controls, where survival percentage was high, less inhibition of both fungi was observed.  
 202 Moreover, a highly significant positive correlation was observed between mortality of *R.*  
 203 *dominica* and infection inhibition of *A. flavus* or *A. parasiticus*, and mortality of *S. oryzae* and  
 204 infection inhibition of *A. parasiticus*. There was an indirect effect of insecticides on the  
 205 inhibition of both fungi. Insecticides caused mortality of the insect species which ultimately  
 206 resulted in infection inhibition of fungi. One most probable reason could be that increased  
 207 mortality of insects resulted in reduced movement and seed coat damage which ultimately lead to  
 208 reduced infection of the fungi. After the harvest of crops, temperature and humidity play an  
 209 important role in the growth of toxigenic fungi and insects in the storage ecosystem [30]. Stored

210 insect pests in the granary ecosystem move for breeding and feeding purposes from one point  
 211 another and contribute the dispersal of viable spores of fungi of various species, including *A.*  
 212 *flavus* and *A. parasiticus*, which are carried on the insect body surface or deposited in their frass  
 213 [30]. During feeding in stored commodities, insects break the seed coat of grains, which is a  
 214 natural barrier to fungal growth, and ultimately promote easy spread of fungal spores [11].  
 215 Hence, in the present study fungi infection was more in those treatments where the survival rate  
 216 of insects was high, most probably due to a high rate of feeding and movement activities.  
 217 Dispersal of fungal spores in storage commodities usually depends on the type of insect species  
 218 present in those commodities [31]. During storage, the invasion by a variety of insects causes  
 219 losses to storage grains and act as mechanical vectors of viable fungal spores [20]. If storage  
 220 grains left untreated, insects within the granary ecosystem can create local conditions of  
 221 humidity and temperature that favor the rapid growth of fungi, resulting in deterioration of the  
 222 grain mass and production of mycotoxins [32]. The results of the correlation in the present study  
 223 are in accordance with those of Lamboni and Hell [31] who reported a significant and positive  
 224 association between the activity of *Sitophilus* sp. and *A. parasiticus*. Similarly, Nesci et al. [30]  
 225 reported weak involvement of *Sitophilus* sp. in the distribution of *A. flavus* spores in stored  
 226 maize whereas *R. dominica* was more susceptible to fungal contamination and spread. From the  
 227 results it is assumed that if the storage commodities left untreated and have viable spores of *A.*  
 228 *flavus* and *A. parasiticus*, and *R. dominica* and *S. oryzae* infestation, there would be more  
 229 chances of spread of the said fungi.  
 230 Since the insecticides tested in the present study are not recommended yet as grain protectants in  
 231 Pakistan, further studies should be conducted to assess their long term efficacy or persistence  
 232 against different stored pests across different stored grain commodities. Spinosad and

233 thiamethoxam proved very effective against *R. dominica* and the subsequent infection inhibition  
234 of both aflatoxin producing fungi than the rest of the insecticide tested. In granary systems where  
235 *R. dominica* is the sole problem, spinosad and thiamethoxam may provide adequate protection.  
236 However, tested insecticides except indoxacarb did not provide adequate control of *S. oryzae*.  
237 Therefore, in storage bins where both insect species coexist, there is a need to evaluate other  
238 insecticides or insecticide combinations which can provide adequate protection against insect  
239 pests and subsequent fungal infection. In conclusion, the results of the present study provide  
240 baseline data for the management of aflatoxigenic fungi by controlling *R. dominica* and *S. oryzae*  
241 using biorational insecticides. Whether this is a practical solution in granary systems for a long  
242 time needs to be further characterizing in future studies.

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## References

1. Bennett JW, Klich M (2003) Mycotoxins. *Clinical Mycobiol Rev* 16: 479-516.
2. Horn BW (2007) Biodiversity of *Aspergillus* section *Flavi* in the United states; A review. *Food add Contam* 24: 1088-1101.
3. Patten RC (1981) Aflatoxins and disease. *Am J Trop Med Hyg* 30: 422- 425.
4. Chao TC, Maxwell SM, Wong SY (1991) An outbreak of aflatoxicosis and boric acid poisoning in Malaysia: a clinicopathological study. *J Pathol* 164: 225-233.
5. Lacey J (1988) The microbiology of cereal grains from areas of Iran with a high incidence of oesophageal cancer. *J Stored Prod Res* 24: 39-50.
6. Desjardins AE, Manandhar G, Plattner RD, Maragos CM, Shrestha K, McCormick SP (2000) Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect traditional processing method on mycotoxin levels. *J Agric Food Chem* 48: 1377-1383.
7. Galvano F, Piva A, Ritieni A, Galvano G (2001) Dietary strategies to counteract the effect of mycotoxins: a review. *J Food Protec* 64: 120 – 131.
8. Philip TW, Throne JE (2010) Biorational approaches for managing stored-product insect. *Annu Rev Entomol* 55: 375-39.
9. Birck NMM, Lorini I, Scussel VM (2006) Fungus and mycotoxins in wheat grain at post harvest. In: 9<sup>th</sup> International Working Conference on Stored Product Protection: 198-205.
10. Santos JP, Mantovani EC (1997) Perdas de grãos do milho pré-colheita, colheita, transporte e armazenamento. *Sete Lagoas, Embrapa/CNPMS*, 6p.
11. Setamau M, Cardwell KF, Schulthess F, Hell K (1998) Effect of insect damage to maize ears, with special reference to *Mussidia nigrivenella* (Lepidoptera: Pyralidae) on *Aspergillus flavus* (Deuteromycetes: Monoliales) infection and aflatoxin production in maize before harvest in the Republic of Benin. *J Econ Entomol* 91: 433-439.
12. Pimentel D. 1991. World resources and food losses to pests 37, pp.5-11.
13. Barra P, Nesci A, Etcheverry M (2013) In vitro compatibility of natural and food grade fungicide and insecticide substances with *Purpureocillium lilacinum* and their effect against *Aspergillus flavus*. *J Stored Prod Res* 54: 67-73.
14. Gurusubramanian G, Rahman A, Sarmah M, Roy S, Bora S (2008) Pesticide usage pattern in tea ecosystem, their retrospects and alternative measures. *J Environ Biol* 29: 813-826.
15. Hazarika LK, Bhuyan M, Hararika BN (2009) Insect pest of tea and their management. *Annu Rev Entomol* 54: 267-284.
16. Cranham JE (1996) Tea pest and their control. *Annu Rev Entomol* 11: 419-514.
17. Vayias BJ, Athanassiou CG, Milonas DN, Mavrotas C (2010) Persistence and efficacy of spinosad on wheat, maize and barley grains against four major stored product pests. *Crop Protect* 29: 496–505.
18. Arthur FH, Yue B, Wilde GE (2004) Susceptibility of stored-product beetles on wheat and maize treated with thiamethoxam: effects of concentration, exposure interval, and temperature. *J Stored Prod Res* 40: 527-546.



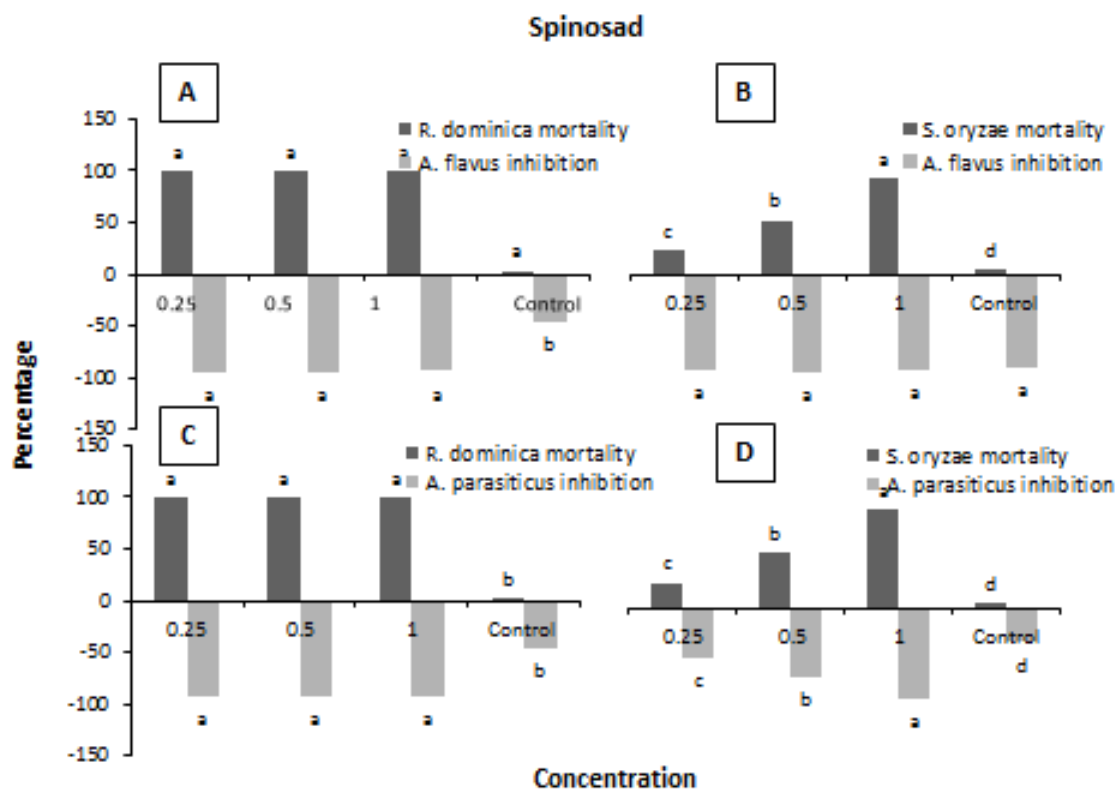
19. Athanassiou CG, Arthur FH, Kavallieratos NG, Throne JE (2011) Efficacy of spinosad and methoprene, applied alone or in combination, against six stored-product insect species. *J Pest Sci* 84:61–67.
20. Ferreira-Castro FL, Potenza MR, Rocha LO, Correa B (2012) Interaction between toxigenic fungi and weevils in corn grain samples. *Food Control* 26: 594-600.
21. Krishnamurthy YL, Shashikala JB, Naik S (2008) Antifungal potential of some natural products against *Aspergillus flavus* in soybean seeds during storage. *J Stored Prod Res* 44: 305– 309.
22. Analytical Software (2005). Statistix Version 8: 1: User's manual. Analytical Software, Tallahassee, Florida.
23. Athanassiou CG, Kavallieratos NG, Chintzoglou GJ (2008) Effectiveness of spinosad dust against different European populations of the confused flour beetle, *Tribolium confusum* Jacquelin du Val. *J Stored Prod Res* 44:47–51.
24. Athanassiou CG, Kavallieratos NG, Yiatilis AE, Vayias BJ, Mavrotas CS, Tomanovic Z (2008) Influence of temperature and humidity on the efficacy of spinosad against four stored grain beetle species. *J Insect Sci* 8:60.
25. Fang L, Subramanyam BH, Arthur FH (2002). Effectiveness of spinosad on four classes of wheat against five stored product insects. *J Econ Entomol* 95: 640–650.
26. Nayak MK, Daglish GJ, Byrne VS (2005) Effectiveness of spinosad as a grain protectant against resistant beetle and psocid pests of stored grain in Australia. *J Stored Prod Res* 41:455–467.
27. Zhang L, Greenberg SM, Zhang Y, Liu TX (2011) Effectiveness of thiamethoxam and imidacloprid seed treatments against *Bemisia tabaci* (Hemiptera: Aleyrodidae) on cotton. *Pest Manag Sci* 67: 226-232.
28. Daglish GJ, Nayak MK (2012) Potential of the neonicotinoid imidacloprid and the oxadiazine indoxacarb for controlling five coleopteran pests of stored grain. *Insect Sci* 19: 96-101.
29. Khan HAA, Shad SA, Akram W (2013) Resistance to new chemical insecticides in the house fly, *Musca domestica* L., from dairies in Punjab, Pakistan. *Parasitol Res* 112: 2049-2054. doi:10.1007/ s00436-013-3365-8. PubMed: 23456023.
30. Nesci A, Barra P, Etcheverry M (2011) Integrated management of insect vectors of *Aspergillus flavus* in stored maize, using synthetic antioxidants and natural phytochemicals. *J Stored Prod Res* 47: 231-237.
31. Lamboni Y, Hell K (2009) Propagation of mycotoxigenic fungi in maize stores by post-harvest insects. *Intl J Trop Insect Sci* 29: 31–39.
32. Sinha KK, Sinha AK (1992). Effect of *Sitophilus oryzae* infestation on *Aspergillus flavus* infection and aflatoxin contamination in stored wheat. *J Stored Prod Res* 28: 211-219.
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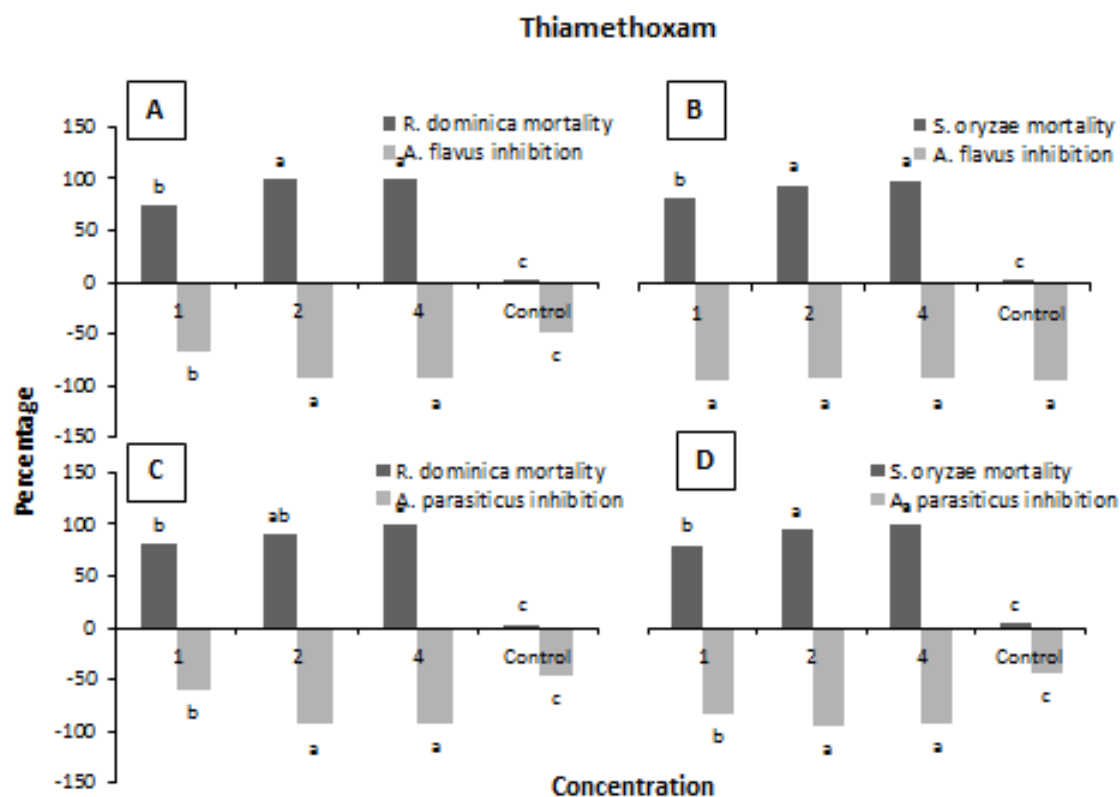
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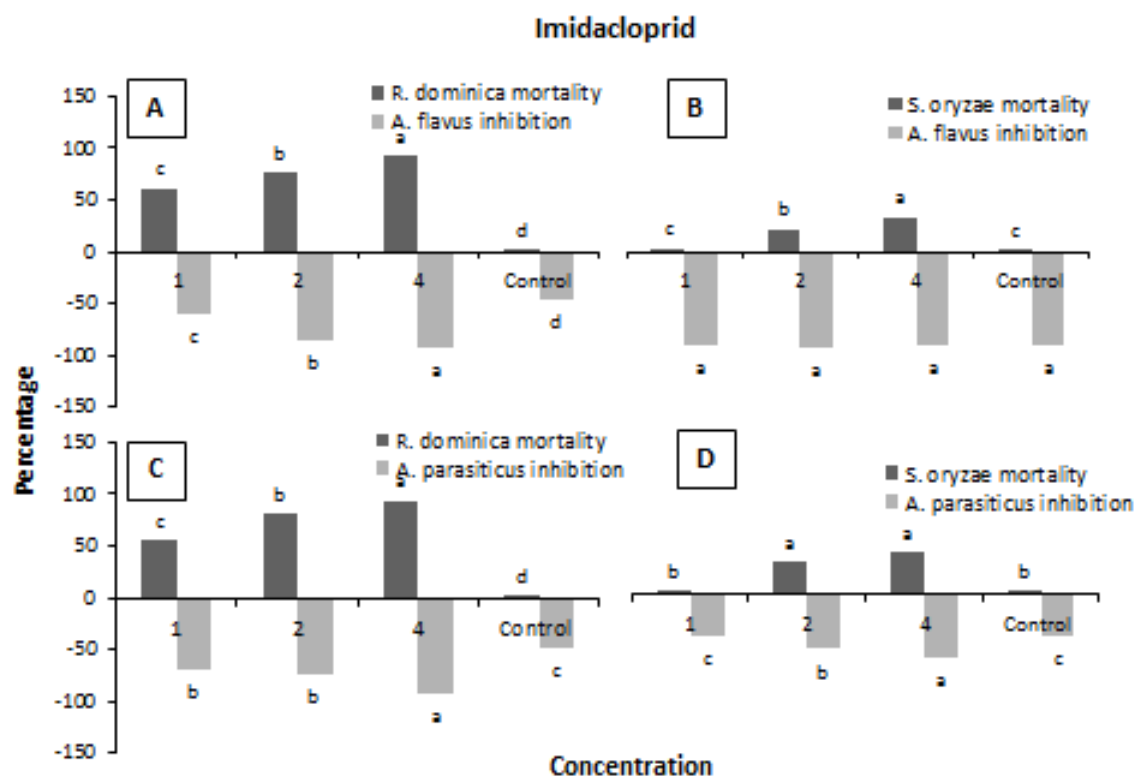
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**Figure 1.** Effect of different concentrations of spinosad on insects' mortality and fungal infection inhibition. Data bars of specific color with different letters are significantly different; in all cases  $df=3,16$ , Tukey's HSD test at  $p<0.05$

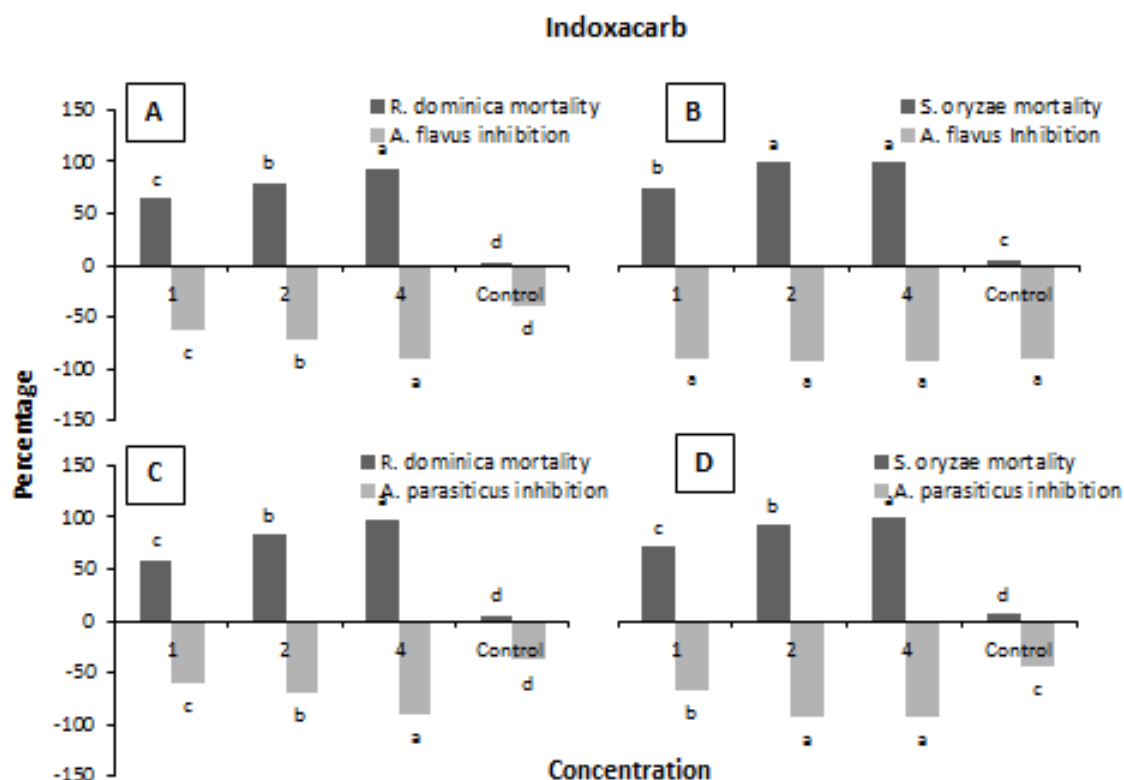


**Figure 2.** Effect of different concentrations of thiamethoxam on insects' mortality and fungal infection inhibition. Data bars of specific color with different letters are significantly different; in all cases  $df=3, 16$ , Tukey's HSD test at  $p<0.05$



345

346 **Figure 3.** Effect of different concentrations of imidacloprid on insects' mortality and fungal  
 347 infection inhibition. Data bars of specific color with different letters are significantly different; in  
 348 all cases df=3, 16, Tukey's HSD test at  $p < 0.05$



**Figure 4.** Effect of different concentrations of indoxacarb on insects' mortality and fungal infection inhibition. Data bars of specific color with different letters are significantly different; in all cases df = 3, 16, Tukey's HSD test at  $p < 0.05$

**Table 1.** correlation analysis between insects' mortality and fungal infection inhibition

	<i>Aspergillus flavus</i>	<i>Aspergillus parasiticus</i>
<i>Ryzopertha dominica</i>	0.93**	0.87**
<i>Sitophilus oryzae</i>	0.18 ns	0.96**

## Figure 1(on next page)

figure of insecticide fungus paper1

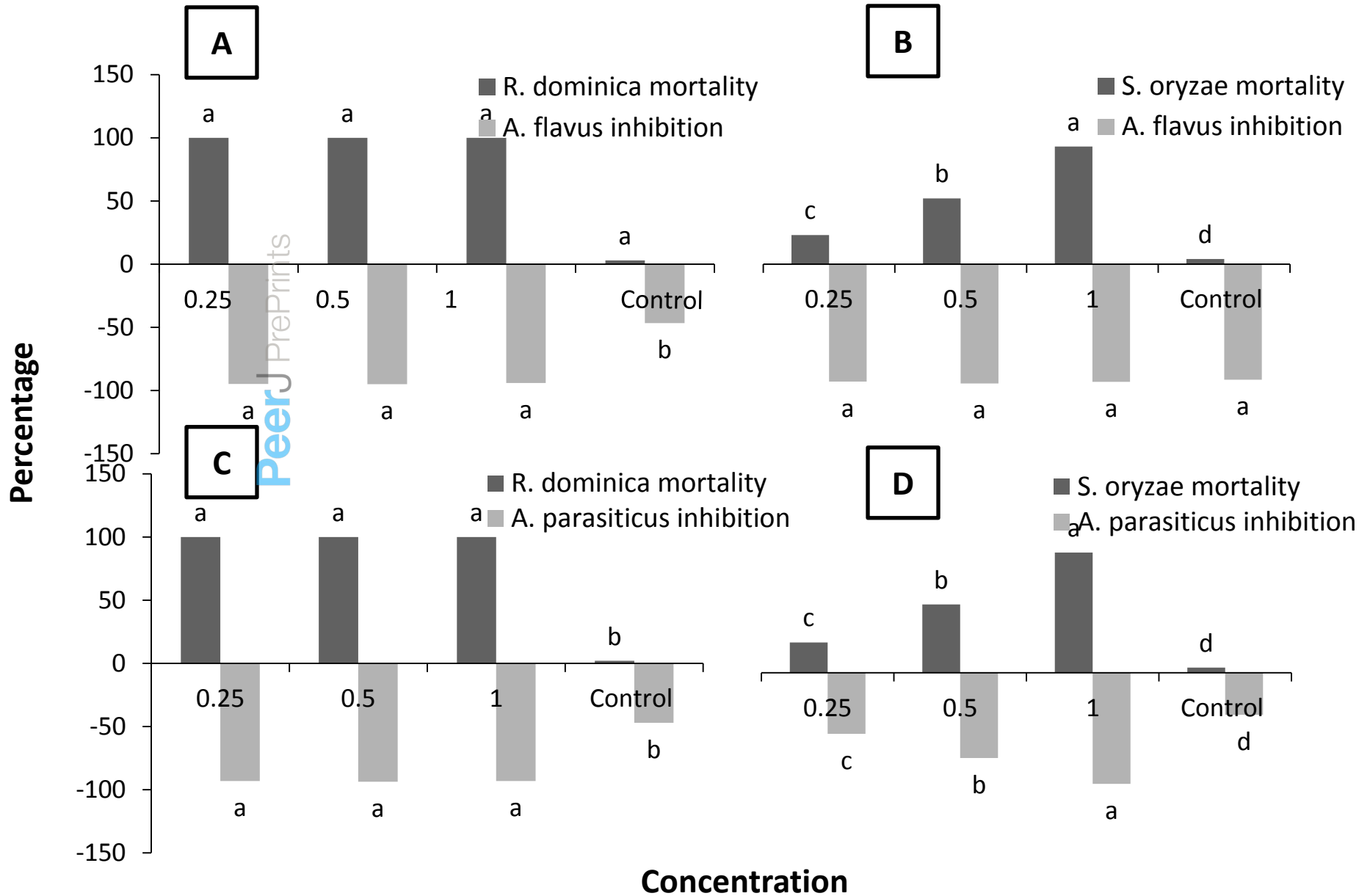
**Figure 1 (Spinosad).** Effect of different concentrations of spinosad on insects' mortality and fungal infection inhibition. Data bars of specific color with different letters are significantly different; in all cases  $df=3,16$ , Tukey's HSD test at  $p<0.05$  **Figure 2 (Thiamethoxam).**

Effect of different concentrations of thiamethoxam on insects' mortality and fungal infection inhibition. Data bars of specific color with different letters are significantly different; in all

cases  $df=3, 16$ , Tukey's HSD test at  $p<0.05$  **Figure 3 (Imidacloprid).** Effect of different concentrations of imidacloprid on insects' mortality and fungal infection inhibition. Data bars of specific color with different letters are significantly different; in all cases  $df=3, 16$ , Tukey's HSD test at  $p<0.05$  **Figure 4 (Indoxacarb).**

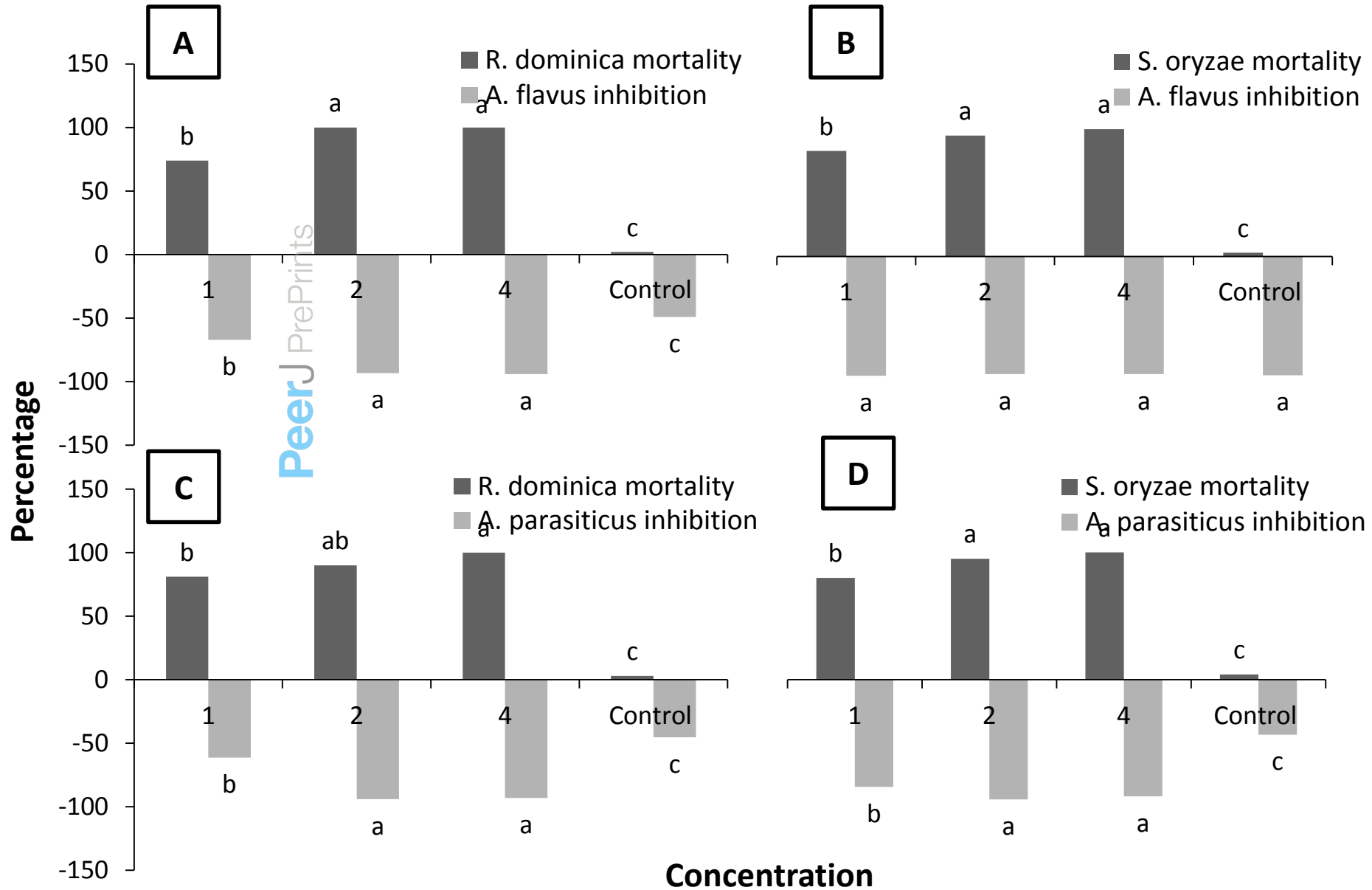
Effect of different concentrations of indoxacarb on insects' mortality and fungal infection inhibition. Data bars of specific color with different letters are significantly different; in all cases  $df = 3, 16$ , Tukey's HSD test at  $p<0.05$

# Spinosad

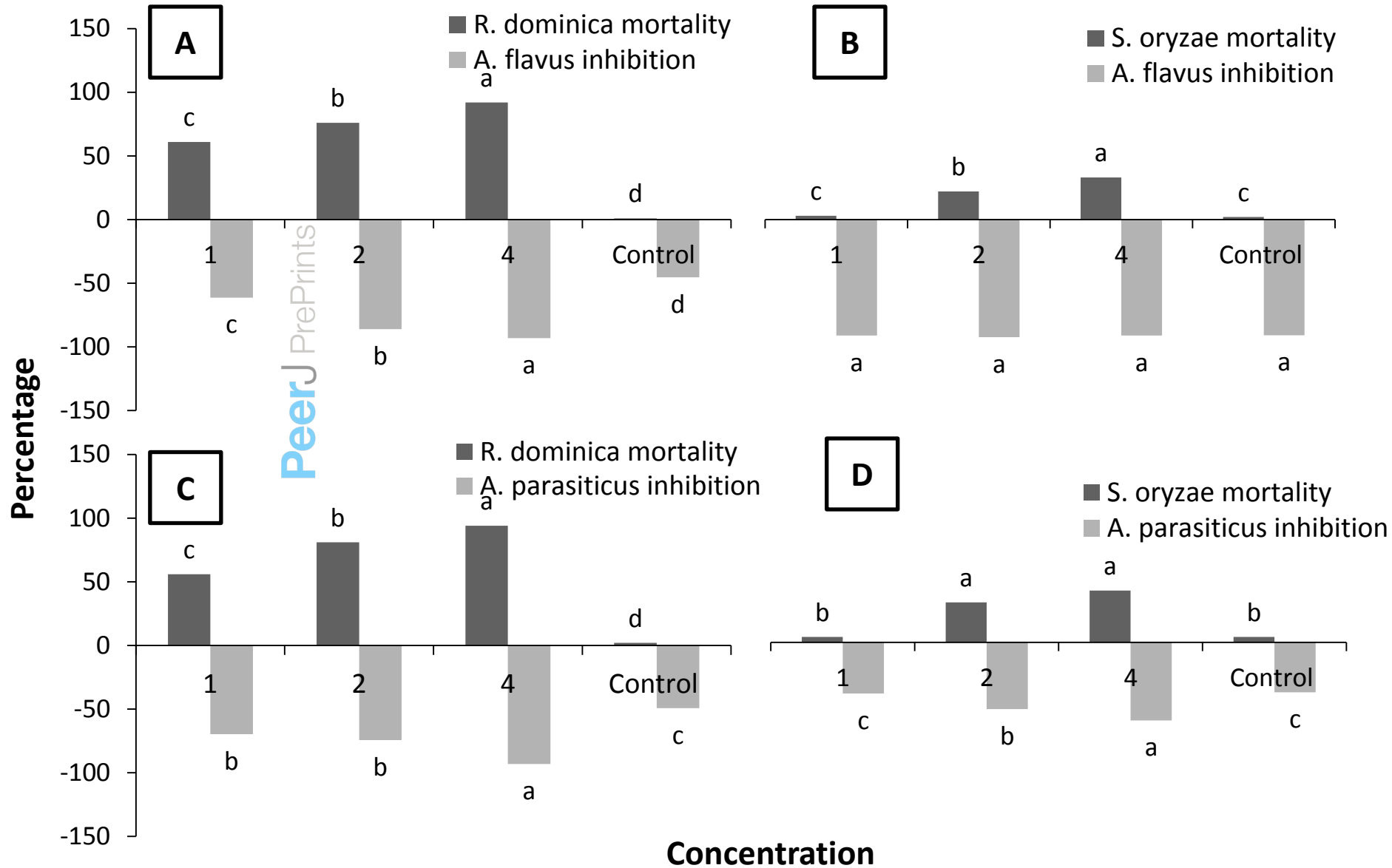




# Thiamethoxam



# Imidacloprid



# Indoxacarb

