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Effect of different biopesticides on mortality and their synergetic effect on the fecundity of *Tribolium castaneum* (Herbst, 1797)

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Abstract : *Tribolium castaneum* is an important stored grain pest. *T. castaneum* not only caused heavy losses in stored grain products, but it also becomes resistant to many pesticides, which makes pests more important. Synergism is an important tool to reduce the risk of the development of resistance. This study was designed to investigate the toxicity, synergism effect, and effect of the combination of different biopesticides on the fecundity of *T. castaneum* Results showed that among six biopesticides (Spinosad, Abamectin, Azadiractin, Rosemary oil, *Metarhizium anisopliae,* and *Verticillium lecanii*) the spinosad, rosemary oil, and *M. anisopliae* exerted maximum percent mortality. When these pesticides were mixed and used to assess the synergistic effect. Results revealed that spinosad + Rosemary oil was the most effective combination, and exerting caused more mortality as compared to spinosad + *M. anisopliae* and rosemary oil + *M. anisopliae.* Results also showed that the number of eggs laid per day was also recorded less in the insects which were exposed to spinosad + rosemary oil as compared to other treatments. The percent reproductive control was about 43% in spinosad + Rosemary oil combination as compared to other treatments. This study showed that the use of different pesticides with a different mode of action in small concentration can be more effective than a single pesticide with high concentration. This technique will effectively deal with pesticide resistance and also economical for the store owners.

Keywords : Bio-pesticides, Botanical pesticides, Entomopathogenic fungi, Fecundity, Percent Reproduction Control, *Tribolium castaneum*.

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Introduction

Worldwide more than 2 billion tons of different grains, including cereals, oilseeds, and pulses, are produced per year to meet the nutritional requirements of humans and other domesticated animals. The storage of these grains is also very important for a long term food availability in case of famine and other natural calamities[1]. Post-harvest losses cannot be assessed accurately due to insufficient reports. In developed countries with well-managed storage conditions, the losses were estimated by up to 1-2%, while in developed countries with poor management, the losses are 20 to 50 % [2,3]. Stored grains are susceptible to the attack of a variety of insect and mite pests. Stored-product insects pest have an economic influence on stored bulk grain and processed commodities[4].

Tribolium castaneum was known as red flour beetle, which is believed to be of Indo-Australian origin [5] but is currently a worldwide insect pest of great economic significance in stored product environments [6,7]. Previous studies showed that adults of *T. Castaneum* in uncontrolled and optimum conditions could cause 50% losses in stored grains[7].

Some medicinal and spice plant contains “secondary plant metabolites” which may act as kairomones, allomones, stimulants or deterrents of feeding and oviposition, and as antifeedants, insecticides and insect hormone mimic. Many plant allelochemicals, including azadirachtin, nicotine, pyrethrins, and rotenoids, have been developed as commercial insecticides[8,9]. Entomopathogenic fungi are considered promising microbial control agents for the control of post-harvest insects pest, and their evaluation for this purpose has lately attracted a significant amount of research. They are naturally occurring, environmentally safe organisms that infect insects by contact. Insect fungal pathogens have a broad spectrum of hosts, can be mass-produced easily, rapidly, and economically and can be applied with the same technical means as conventional contact insecticides[10]. The use of entomopathogenic fungi and botanicals is emphasized due to their environmental-friendly nature to control stored product insects and mites in the laboratory and field-scale trials[11].

The stored grain pests almost became resistant to synthetic chemicals due to the unfair use of insecticides, especially phosphine[12,13]. The incidence of pesticide resistance is a growing problem in stored-product protection. More than 500 species of mites and insect pests are reported to resistant against one or more than one pesticides[14]. Stored product insect pests were found to be resistant against several insecticides including bioresmethrin, carbaryl, chlorpyrifos, chlorpyrifos-methyl, cyanophos, cyfluthrin, cyhalothrin, cypermethrin, DDT, deltamethrin, diazinon, dichlorvos, ethylene dibromide, fenitrothion, lindane, malathion, methyl bromide, permethrin, phosphine, phoxim, pirimiphos-methyl, promecarb, propoxur, pyrethrins, temephos, tetrachlorvinphos[15]. Previously many studies reported the synergistic effect of pesticides against several insects of stored products had shown synergism. The combination of different modes of action could also reduce the development of resistance[16].

In this specific study, different bio-pesticides were used to assess the toxicity of these insecticides against *T. castaneum*. Three highly effective insecticides drawn on the basis of individual pesticide toxicity bioassay and were combined and subjected to assess the synergistic effect of these insecticides on mortality and the fecundity of *T. castaneum.*

Materials and Methods

Insect rearing

Wild type population of *Tribolium castaneum* was collected different stored products infected with red flour beetle from different storage facilities. Collected insects were reared under laboratory conditions in an incubator at 30±2 ^{o}C temperature and 60±5% relative humidity on wheat flour obtained from sterilized wheat grains. The insect was reared for more than three generations before use for experimentation.

Insecticides

Six different bio-pesticides formulations were used in this experiment. Commercially available formulations were purchased from different pesticide stores. These pesticides were diluted for different concentrations at the rate of 0.1 mg/L, 0.5 mg/L, 1.0 mg/L and 2.0 mg/L in water. The selection of concentrations was made on the basis of preliminary experiments. The information regarding pesticides, their recommended dose, and manufacturer information is provided in Table 1.

Table 1. Information regarding pesticide used in experimentation

Trade name	Active ingredient	Company	Formulation
Tracer®	Spinosad	Dow Agro Sciences USA	80ml/Acre
Abamectin®	Abamectin	Arysta Life Science	18 g/L
Margosom®	Azadiractin	Agri-Life	2 ml/ L
EcoTrol™	Rosemary oil	KeyPlex	100 g/L
Pacer®	*Metarhizium anisopliae*	Agri-Life	5 g/L
Mealikil®	*Verticillium lecanii*	Agri-Life	5 g/L

Toxicity assessment bioassay of different pesticides against *Tribolium castaneum*

For the assessment of the toxicity of different pesticides, the experiment was conducted in a 9cm diameter petri dish. A filter paper of 9 cm diameter was socked in a single dilution for 2 minutes and air-dried for 15 min on room temperature. This filter paper was then laid down in the bottom of the petri dish. 20 (five days old) adults were released into the petri dishes. These insects were exposed to the pesticides for 6 hours on the basis of preliminary experiments and shifted into new jars containing wheat flour and covered with a black muslin cloth to prevent insects from escaping. After 3, 7, and 14 days, flour was sieved, and dead insects were counted. Each treatment was replicated four times.

Synergistic effect of different pesticide

After determination of toxicity of individual insecticides, three pesticides **(i)** one from chemical pesticides (Spinosad and Abamectin) **(ii)** one from botanical pesticides (Azadiractin and Rosemary oil) **(iii)** one from entomopathogenic fungal formulations (*Metarhizium anisopliae* and *Verticillium lecanii*) were selected on the basis of their high percent mortality. For the synergistic effect, the concentrations exerting 50% mortality in the first 7 days were selected. The pesticide concentrations were used in a 1:1 ratio in three different combinations (1) A+B (2) B+C (3)A+C. All the combinations were used to assess the mortality by the methods mentioned above.

Fecundity of *Tribolium castaneum*

To assess the effect of these pesticides on the fecundity of *Tribolium castaneum*, 500µl of each concentration was mixed with 50g of whole wheat flour. Control was established by mixing 500µl acetone with 50g of wheat flour. *T. Castaneum* adults were sexed, as mentioned by[17]. 5 females and 5 males (5 days old) were released in each jar. Each treatment was replicated 4 times. All the jars were kept in a controlled environmental chamber at 30±2 °C temperature and 55±5% R.H. After 3days, interval wheat flour was sieved with a 60-mesh sieve and the number of eggs collected in sieve were counted for next 33 days. The percent reproductive control (P.R.C.) was calculated as mentioned by Rizvi[18].

$$P.R.C = \frac{\text{Eggs laid by the female in control} - \text{Eggs laid by the female in treatement}}{\text{Eggs laid by the female in treatement}} \times 100$$

Statistical analysis

The corrected mortality was calculated by the Abbott formula; the data transformation was done by [$\log_{10}(\sqrt{n})$], where n is the value of corrected mortality. Data were analyzed by univariate analysis of variance, and the means were separated using Tukey test ($P < 0.05$). Standard error bars computation and performance of statistical analysis were done by using the SPSS statistical software package version 20. Graphical work was performed with SigmaPlot 20.0.

Results

Toxicity of different pesticides against *Tribolium castaneum*

Results Fig. 1 the mortality due to exposure of spinosad was significantly increased as the concentration increases ($F=102.61$; df=3; $P<0.01$) and days of application ($F=68.83$; df=2; $P<0.01$) (Fig. 1). The maximum (98.68±1.32%) mortality was observed at 2 g/L after 14 days of application. The 50% mortality was observed at 0.5 g/L after 7 days of application (55±3.08%) (Table 2). Mortality due to exposure of abamectin was also significantly affected by the variation in concentrations ($F= 87.39$; df=3 ; $P<0.01$) and days of application ($F=74.17$; df= 2; $P<0.01$) (Fig.1). The 50% mortality was achieved after 7 days of application at 1 g/L (59.54±4.42%), while maximum mortality was observed at 2 g/L after 14 days of interval (93.68±2.38%) (Table 2). The exposure of azadiractin at 1 g/L gives 58.22±3.12% mortality after 7 days of exposure. Maximum mortality was observed after 14 days of the application when azadiractin was applied at the rate of 2 g/L (81.84±3.34%) (Table 2). The variation in concentration and Post-application time have significant effect on mortality ($F= 71.08, 41.99$; df= 3, 2 ; $P<0.01$, respectively) (Fig. 1). Results in Table 2 also revealed that rosemary oil exerts 89.67±1.99% percent mortality in the response of application of 2g/L after 14 days, the shortest time to attain 50% mortality was 7 days at 1 g/L. Overall the variation in concentration and time after application have significant impact on *T. castaneum* mortality ($F= 84.23,66.74$; df=3,2 ; $P<0.01$) (Fig. 1). The application of entomopathogenic fungal formulations of *M. anisopliae* attained 50.66±2.14% mortality in 7 days at 1 g/L pesticide, and *V. lecanii* attained 61.45±2.05% mortality after 7 days of application of 2g/L pesticide (Table 2). The increase in concentrations and after application time have significant effect on mortality in *M. anisopliae* ($F= 59.55, 98.82$; df=3,2 ; $P<0.01$) and *V. lecanii* ($F=57.77, 34.27$; df=3,2 ; $P<0.01$ respectively) (Fig. 1). On the basis of the above results spinosad at the rate of 0.5 g/L, rosemary oil at the rate of 1 g/L and *M. anisopliae* at the rate of 1 g/L were selected. These pesticides were mixed in three different combinations (1) Spinosad+ *M. anisopliae* (2) Rosemary oil + *Metarhizium anisopliae* (3) Spinosad + Rosemary oil and subjected to assess their synergistic effect.

Table 2. Percent mortality of different pesticides against *Tribolium castaneum* exposed to different concentrations.

Chemical	Conc.	DAP	Mortality (%) Mean±SE	Chemical	Conc.	DAP	Mortality (%) Mean±SE
Spinosad	**0.1 g/L**	**3 days**	20.0±2.04	**Abamectin**	**0.1 g/L**	**3 days**	13.75±2.39
		7 days	26.91±2.40			**7 days**	25.20±3.35
		14 days	29.74±4.04			**14 days**	36.71±1.18
	0.5 g/L	**3 days**	35.0±3.54		**0.5 g/L**	**3 days**	26.25±3.75
		7 days	55.0±3.08			**7 days**	37.96±2.39
		14 days	66.18±1.78			**14 days**	51.78±3.82
	1.0 g/L	**3 days**	38.75±2.39		**1.0 g/L**	**3 days**	40.0±2.04
		7 days	62.83±1.04			**7 days**	59.54±4.42
		14 days	80.46±2.63			**14 days**	75.86±2.74
	2.0 g/L	**3 days**	41.25±2.39		**2.0 g/L**	**3 days**	46.25±2.39
		7 days	76.91±3.26			**7 days**	72.04±3.60
		14 days	98.68±1.32			**14 days**	93.68±2.38
Azadiractin	**0.1 g/L**	**3 days**	11.25±2.39	**Rosemary oil**	**0.1 g/L**	**3 days**	11.25±2.39
		7 days	21.38±4.15			**7 days**	22.83±1.66
		14 days	25.92±3.55			**14 days**	31.18±2.18
	0.5 g/L	**3 days**	25.0±2.04		**0.5 g/L**	**3 days**	27.50±3.23
		7 days	39.14±2.78			**7 days**	37.96±2.39
		14 days	55.92±2.92			**14 days**	57.24±2.71
	1.0 g/L	**3 days**	36.25±2.39		**1.0 g/L**	**3 days**	35.0±2.04
		7 days	58.22±3.12			**7 days**	55.66±2.56
		14 days	71.51±3.05			**14 days**	74.08±1.84
	2.0 g/L	**3 days**	45.0±4.56		**2.0 g/L**	**3 days**	47.50±3.23
		7 days	69.67±3.39			**7 days**	65.86±2.21
		14 days	81.84±3.34			**14 days**	89.67±1.99
Metarhizium anisopliae	**0.1 g/L**	**3 days**	12.70±1.57	***Verticillium lecanii***	**0.1 g/L**	**3 days**	08.75±2.39
		7 days	25.26±2.74			**7 days**	16.51±3.60
		14 days	40.99±1.72			**14 days**	28.09±2.94
	0.5 g/L	**3 days**	21.58±2.58		**0.5 g/L**	**3 days**	17.50±3.23
		7 days	29.14±3.22			**7 days**	25.59±3.43
		14 days	43.49±2.75			**14 days**	39.67±2.89
	1.0 g/L	**3 days**	27.89±3.35		**1.0 g/L**	**3 days**	35.0±3.54
		7 days	50.66±2.14			**7 days**	43.55±2.31
		14 days	64.14±1.76			**14 days**	58.95±2.23
	2.0 g/L	**3 days**	36.64±2.06		**2.0 g/L**	**3 days**	43.75±3.15
		7 days	62.04±3.14			**7 days**	61.45±2.05
		14 days	88.55±4.69			**14 days**	80.66±2.73

Conc. = Concentrations; DAP= Days after exposure

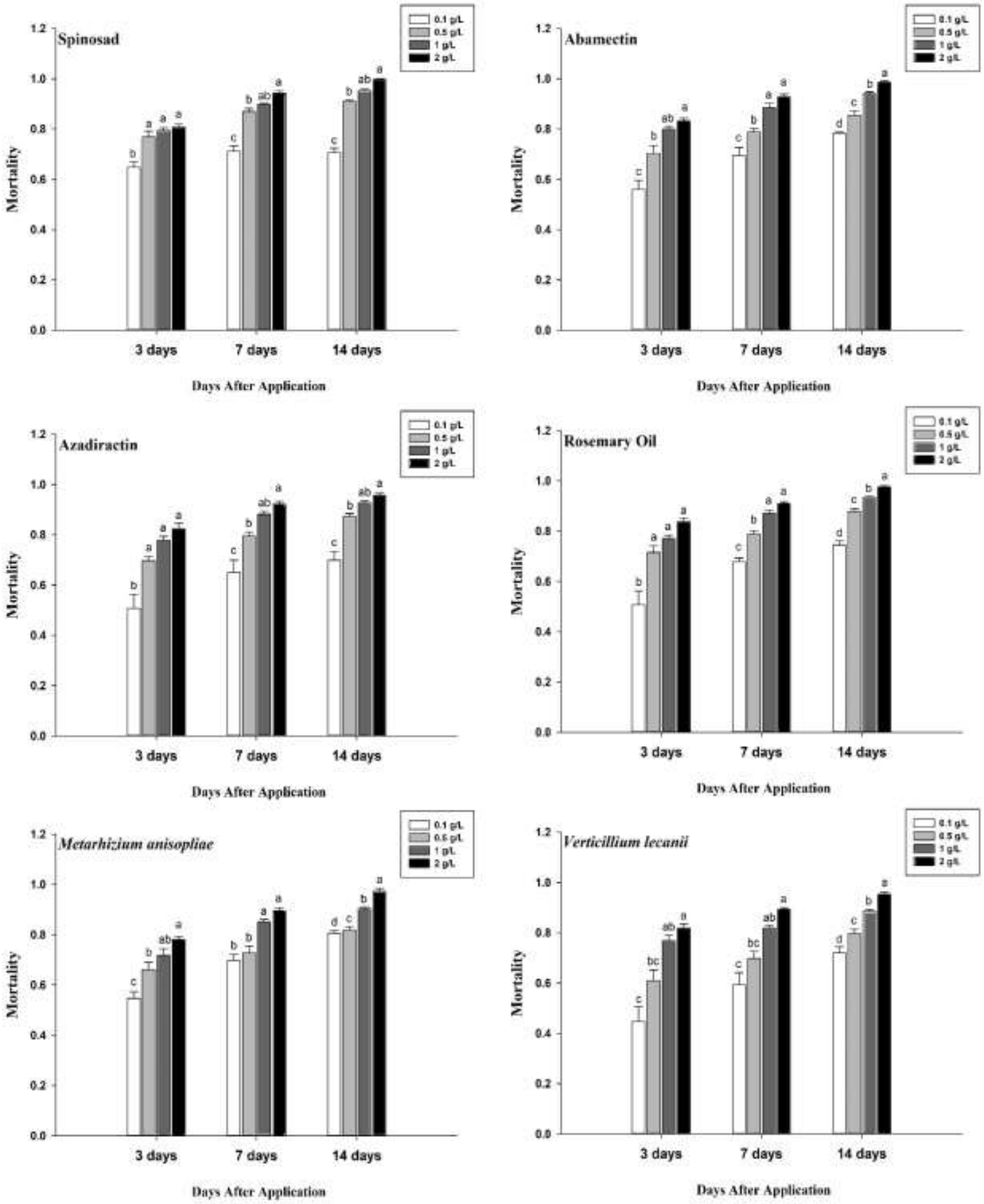

Fig. 1. Mortality of different pesticides after 3, 7, and 14 days. Each bar is the mean value of four replications treated with 0.1, 0.5, 1.0, and 2.0 mg/L. Error bars are showing a standard deviation of mean at a 95% confidence interval. The same lettering is showing the non-significant difference between means.

Synergistic effects of three pesticides against *Tribolium castaneum*

When the different combinations of pesticides subjected for assessment of mortality due to synergistic effect, results in Fig. 2 showed that all the combinations (f= 12.81; df= 2; p<0.01) and days (f= 145.20; df= 2; p<0.01) exerted a significant effect on the mortality of *T. castaneum*. Results (Table 3) also revealed that the combination of spinosad + rosemary oil gives 100% mortality of adults after 14 days of application, while this percentage was 84.54±2.26 and 83.22±3.43 in Spinosad+ *M. anisopliae* and Rosemary oil + *M. anisopliae*, respectively.

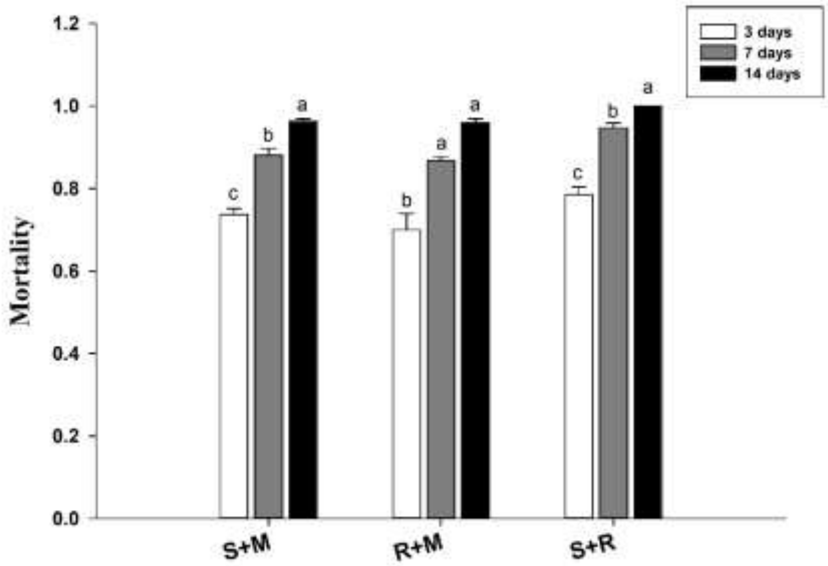

Fig. 2. Mortality of three pesticide combinations (S+M= Spinosad+*Metarhizium anisopliae*; R+M= Rosemary oil+*Metarhizium anisopliae*; S+R= Spinosad+Rosemary oil) after 3, 7 and 14 days. Each bar is the mean value of four replications. Error bars are showing a standard deviation of mean at a 95% confidence interval. The same lettering is showing the non-significant difference between means.

Table 3: Percent mortality of different pesticide combinations

Combinations	**DAP**	**Mortality (%) Mean±SE**
Spinosad+*Metarhizium anisopliae* (S+M)	**3 days**	30.0±2.04
	7 days	58.22±4.25
	14 days	84.54±2.26
Rosemary oil+*Metarhizium anisopliae* (R+M)	**3 days**	26.25±4.27
	7 days	54.34±2.61
	14 days	83.22±3.43
Spinosad+Rosemary oil (S+R)	**3 days**	37.50±3.23
	7 days	78.49±4.25
	14 days	100±0.00

DAP= Days after exposure

Fecundity of *Tribolium castaneum*

When whole wheat flour was treated with different combinations of pesticides. Results that the number of eggs/day was significantly reduced in spinosad + rosemary oil treatment (4.18±0.18 eggs/day) as compared to that observed in spinosad+ *M. anisopliae* (5.58±0.23 eggs/day) and rosemary oil + *M. anisopliae* (6.11±0.13 eggs/day). The maximum number of eggs observed in control (7.27 eggs/day) (Fig. 3). There was 42.27±3.02% reproduction control observed in spinosad + rosemary oil treatment as compared to that in spinosad+ *M. anisopliae* (23.23±2.89%) and rosemary oil + *M. anisopliae* (15.73±2.81%) (Fig. 4).

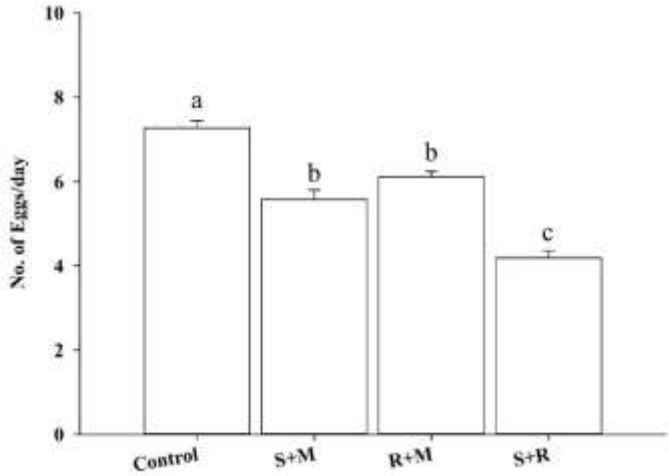

Fig. 3. Number of eggs laid by *Tribolium castaneum* per day. Treated with different pesticide combinations (S+M= Spinosad+*Metarhizium anisopliae*; R+M= Rosemary oil+*Metarhizium anisopliae*; S+R= Spinosad+Rosemary oil) and control. Each bar is the mean value of four replications. Error bars are showing a standard deviation of mean at a 95% confidence interval. The same lettering is showing the non-significant difference between means.

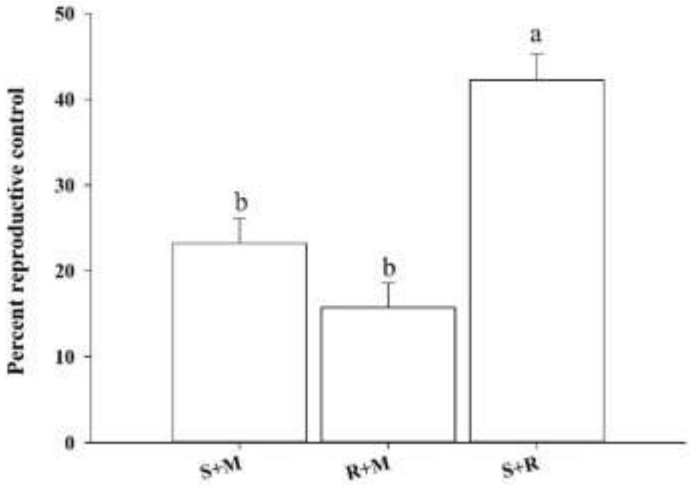

Fig. 4. Percent reproductive control of *Tribolium castaneum*. Treated with different pesticide combinations (S+M= Spinosad+*Metarhizium anisopliae*; R+M= Rosemary oil+*Metarhizium anisopliae*; S+R= Spinosad+Rosemary oil). Each bar is the mean value of four replications. Error bars are showing a standard deviation of mean at a 95% confidence interval. The same lettering is showing the non-significant difference between means.

Discussion

Insects on their own have a great potential to detoxify repeatedly applied toxic compounds by increasing the enzymatic metabolism of those compounds[19,20]. Synergists help to fight against such hypermetabolism of insecticides, hence increase their real efficacy at lower doses against resistant insects [21,22].

Several studies reported the synergistic effect of different pesticides against stored grain pests. Ziaee[23] reported the synergistic effect of *Carum copticum* essential oil on diatomaceous earth tested against *Sitophilus granarius* and *T. confusum.* The synergistic effect of spinosad with three botanical powders of *Aframomum melegueta*, *Eugenia aromatica,* and *Piper guineense* against *T. castaneum* was reported by Babarinde[24]. The synergistic effect of piperonyl butoxide and emamectin benzoate was assessed to determine the resistance level in *T. castaneum* Herbst by Awan [25].

Metarhizium anisopliae was tested against black bug (*Scotinophara courctata*), and observations revealed that the black bug population was significantly reduced in the treated rice field due to the application of entomophagous fungi[26]. Batta[27] used *M. anisopliae* against *Rhyzopertha dominica* in *Cicer arietinum*. Results showed that exposure to the early stages of pests could cause a serious reduction in F1 progeny. Conidia suspended in distilled water or formulated in invert emulsion or wheat flour caused 56.7, 93.3, and 86.7% mortality, respectively, 7 days after treatment.

Results of the application of azadiractin against *R. dominica* showed that the application of azadiractin on wheat grains at the rate of 5 mg/Kg reduced the F1 progeny emergence by 98% [28]. Hussain[29] examined six insecticides with novel modes of application such as abamectin (Sure 1.8 EC), spinosad (Tracer 240 SC), indoxacarb (Steward 150 SC), azadirachtin (Nimbokil 60 EC), buprofezin, and polychlorinated petroleum hydrocarbon (Tenekil 100 EC) against *T.castaneum*. Results show that abamectin was the most toxic of all insecticides applied in current study, followed by indoxacarb, spinosad, buprofezin, Tenekil 100 EC, and azadirachtin. These results somehow contradict the results of the present study, which showed that spinosad was the most effective pesticide against *T. castaneum.* Spinosad at the rate of 0.25%, 0.50%, 0.75% and 1.0% was used against *T. castaneum*. The results showed that as the concentration of pesticide increases the mortality of red flour beetle also increases. The mortality of insects in treatment was significantly different as compared to that in control [30]. Andric[31] compared the effect of abamectin and spinosad on *T. castaneum*. The conclusion was drawn that if the spinosad and abamectin applied at the same rate of 5 mg/Kg, the mortality recorded in spinosad exposed insects was 75% while in abamectin was 58%, which showed that spinosad is more toxic for *T. castaneum* as compared abamectin.

Sabbour[32] reported that that *T. castaneum* was susceptible to *N. riley,* but larvae of *T. castaneum* were more tolerant of *V. lecanii.* Das[33] used neem-based insecticide Nimbicidine® against *T. castaneum,* and results showed that insecticide significantly inhibited the hatching, pupation, and adult emergence of the beetle. Khanam[34] reported that the exposure of plant extracts to *T. castaneum* could cause a significant reduction in fecundity, fertility, and oviposition.

Ashraf [35] reported that *M. anisopliae,* the combination of diatomaceous earth and thiamethoxam, was used to assess their mortality, progeny production, mycosis, and sporulation of different stored grain insect pests and reported that the combination of pesticides reduced a mean number of emerged adults.

Conclusion

On the basis of above-mentioned results the conclusion can be drawn that spinosad, rosemary oil and *M. anisopliae* are effective biopesticides against *T. castaneum,* but because of risk of the resistance development if these pesticides used in lower doses and mixed. The synergistic effect gives not only effective control of the adult population but also control the oviposition of *T. castaneum.* For the validation of the results of curent study, there is a need to conduct the experiment in storage places, which can providee a resistance-free control method and also helps to lower the post-harvest storage cost.

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