VOLUME 11 ISSUE 2 2025

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 11, No. 2, 350-360 (2025)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Efficiency of a Novel *Tagetes erecta* Based Biopesticide against *Sitophilus zeamais* Infestation in Stored *Sorghum bicolor*

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Received: 13th August, 2025; **Accepted:** 22nd August, 2025; **Published online:** 29th August, 2025

https://doi.org/10.33745/ijzi.2025.v11i02.032

Abstract: In India, the problem of the optimum food supply to cope with the population has been resolved by the implementation of the Green Revolution by Dr Swaminathan. However, this creates anxiety for human health and the environment due to the excessive use of chemical pesticides and fertilizers in agricultural practices. According to the World Bank's report, post-harvest losses of food grains in India account for approximately 12 to 16 million metric tons per year. About 14 million tons of food grains of Rs 70000 crores, out of which the insects contribute to the tune of Rs. 13000 crores annually. In the agricultural sector and post-harvest preservation practices, organic farming and IPM are the future tools for the next generations. The *Sitophilus zeamais* is a pest that infests grains such as Sorghum, maize, wheat, and rice to a high degree. To acquire the economic and nutritional values of such food grains, biopesticide formulation is vital. In this study bio-pesticide formulation from local plants, *Tagetes erecta*,is shown as an effective method for the preservation of *Sorghum bicolor* from infestation by *Sitophilus zeamais.* It was shown that the extracts of flowers of *Tagetes erecta* in different solvents were active against the growth and survival of *S. zeamais* in stored food grains such as *Sorghum bicolor*.

Keywords: *Sitophilus zeamai*, *Tagetes erecta*, *Sorghum bicolor*, Soxhlet Apparatus, Stored grains, Preservation

Citation: Andhale Atmaram Vitthal: Efficiency of a novel *Tagetes erecta* based biopesticide against *Sitophilus zeamais* infestation in stored *Sorghum bicolor.* Intern. J. Zool. Invest. 11(2): 350-360, 2025.

https://doi.org/10.33745/ijzi.2025.v11i02.032

Introduction

The Green Revolution enhances agricultural commodities due to the use of chemical fertilizers and pesticides. Insecticides or pesticides enhance or safeguard agricultural yields and the quality of agricultural products and decrease labor input (Mathur, 1999). But there is an irresistible evidence that some of these chemicals do pose potential risks to humans and other life forms and unwanted side effects to the environment (Forget, 1993). In the mid-20th century, DDT revolutionized agricultural practices because of its high effectiveness. DDT is credited for managing insect-carrying diseases of malaria, dengue, trench fever, plague, louse-born typhus, dysentery, diarrhea, and many others (Jiang and Wan, 2009). But later on, DDT was banned due to adverse effects on the

environment. Insecticides may be poisonous to man, different animals, and plants if improperly used. Numerous early pesticides and fungicides comprise tremendously toxic heavy metals including arsenic, copper, lead, mercury, and nicotine. Many common insecticides include powerful neurotoxic chemical substances that assault and disable the nervous system and brain (Aktar *et al*., 2009).

Pesticides are frequently taken into consideration as a short, easy, and cheaper solution for controlling weeds, and insect pests in city landscapes and agricultural commodities. However, pesticide use comes at a significant cost. Insecticides have contaminated almost every part of our surroundings. Pesticide residues are discovered in soil and air, and surface and groundwater across the countries. Pesticide infection poses tremendous risks to the environment and non-target organisms, starting from beneficial soil microorganisms to insects, flora, fish, and birds (Knipling, 1953).

Looking at the direction of these types of drawbacks of the usage of conventional pesticide in agricultural practices, it is vital to screen out and increase the new techniques for the development of crop yields and to keep the environment accordingly to conserve biodiversity (Pansare *et al*., 2022). Bio-pesticides are less toxic, more target-specific, eco-friendly, effective with small quantities, decompose quicker compared to conventional insecticides, and cause less pollution (Lewis *et al*., 1997; Mahdi-Pour *et al*., 2012; Pansare *et al*., 2022.

Sorghum bicolor (L.), commonly called Sorghum, or jawari, is an important cereal crop grown globally for food and feed purposes. It is generally cultivated in semi-arid tropics where water is scarce and drought is frequent (Rampho, 2005). The crop is environment-friendly as it is water efficient, requires little or no fertilizers, and is biodegradable (Simonyan *et al*., 2007). Sorghum has an extremely good advantage in both the domestic and international markets due to its growing demand for the manufacturing of meals and feed products, as alcoholic and non-alcoholic liquids (FAO, 1999). Sorghum additionally has a great potential source of starch used as raw material in different industries (Mahai *et al*., 2015). Farmers and grain processors were evolving several conventional practices via trial-and-error methods to keep away from big losses that arise in stored grains due to insect pest infestation (Adejumo and Raji, 2007).

Many insect pests have been the major problem of agriculture in the tropics for a long, as beneficial situations exist for the pest and terrible post-harvest handling facilities, which results in substantial waste of farm produce and hence, considerable loss to the economy (Okonkwo, 2001).

Insect pests damage stored grains significantly which affects quality, nutritive, and viability of grains like sorghum, maize, wheat, and rice (Utono, 2013). Insect pests cause severe damage to cereals stored in traditional storage systems and marketplace stores in a few parts of Nigeria (Suleiman and Rugumamu, 2017).

The maize weevil, *Sitophilus zeamais*, is one of the most destructive stored products pests of grains, cereals, and different processed and unprocessed stored products in sub-Saharan Africa. *S. zeamais* causes qualitative and quantitative harm to stored products, with grain weight loss ranging from 20-90% in untreated saved maize, and the severity of the damage depends on factors that include storage, structural physical, and chemical properties (Rabia and Asghari, 2013). *Sorghum bicolor* is highly infested by *Sitophilus zeamais* at room temperature. To avoid the damage of post-harvest *Sorghum bicolor,* one needs to define or screen out the safety and scientific methods for its preservation.

Tagetes erecta has been documented for various medicinal properties, which include antioxidant, antibacterial, and diuretic activities, anti-inflammatory, wound healing properties, and mosquitocidal efficiency (Varahi Vedam *et al*., 2019). *Tagetes erecta* is used internally for joint

aches, irregular menstruation, belly aches/abdominal pain, and healing. Different medicinal uses of *T. erecta* are as a central nervous system stimulant, antidepressant, antioxidant, antipyretic, antidiabetic, and hypolipidemic agents (Mailafiya, 2003). In Ayurveda, Marigold is taken into consideration as cooling in nature and, as a consequence, balancing for Pitta (pH) as well as Kapha Dosha. Flavonoids, which are present in the flowers of Marigold, are stated to be related to the remedy of most cancers due to their anti-inflammatory qualities (Arefin *et al*., 2015).

Observing challenges of infections of different food grains by *Sitophilus zeamais,* and the medicinal properties of *Tagetes erecta*, one needs to avoid the damage of post-harvest *Sorghum bicolor* and agricultural commodities by screening the eco-friendly and medicinally important pest control materials from natural origins. In this study bio-pesticide formulation from local plants, *Tagetes erecta,* is shown as an effective method for the preservation of *Sorghum bicolor* from infestation by *Sitophilus zeamais.*

Materials and Methods

Non-infected grains of the *Sorghum bicolor* (Jowar) were purchased from the local market and were screened for damaged and infected grains if any. The non-infected grains were washed in clean water and dried. The dry grains were placed in the clean glass bottle. The cap of the bottle was perforated for ventilation. A wet muslin cloth was tied at the mouth of the bottle before capping to maintain the humidity for the survival of the pest.

Sitophilus zeamais species from infected pulses were obtained from the market and were identified at Zoological Survey of India (ZSI), Pune, India. 10 males and 10 females of *Sitophilus zeamais* were released in the bottle containing 500 g of the grains of *Sorghum bicolor* and were allowed to grow. The adult females laid the eggs on the grains. Beetles, when they died, were removed, and the culture was maintained at room temperature. After about 28 days, the young adults have begun to emerge from the grains. The adults hatched were used for the experiment, and some were released in the fresh pulses again to maintain the stock culture.

Flowers of *Tagetes erecta* (Yellow) and *Tagetes erecta* (Orange) were collected from the local area and dried in the shade, and then in the oven. The dried flowers were powdered separately in the grinder, and powders were stored in the airtight polyethylene bags. The powder was packed in filter paper and extracted in Soxhlet apparatus in 1/2:10 ratio i.e., 10 g powder in 200 ml of solvent. Flower extracts extracted in Ethanol, Butanol and Aqueous after solvent evaporation were stored in the refrigerator. After 8 h of continuous extraction, the final extract was kept open to evaporate the solvent and finally made it to 100 ml extract solution which is used as stock solution. The stock solution was stored at 4 °C for further use to prepare the working experimental solution. From this stock solution we had made serial dilutions as 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml for experimental setup. These dilutions were made up to 1 ml by adding distilled water.

100 g of the disinfected Sorghum grains were taken in each of the plastic containers. One container was maintained as a control and others were labeled and were used for the experiment. The stock solution was dissolved in respective solvents that is Ethanol, Butanol, and Distilled water to prepare 1 ml working solution of different concentrations i.e., 0.2, 0.4, 0.6, 0.8, 1.0, respectively. 1 ml of working solution in different solvents with different concentrations was added in each experimental container as a dose, which contained 100 g of sorghum grains. Containers were kept open for 48 h in a well-ventilated room to evaporate the ethanol and butanol. After 48 h, 20 beetles (*Sitophilus zeamais*) from stock culture were released in each of the control and experimental containers and those containers were covered up with muslin cloth. The mortality rate was observed for 120 h (5 days) of exposure to different concentrations of plant extracts and was recorded.

Table 1: Effect of ethanol extract of *Tagetes erecta* (yellow flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolor*

Conc. of Stock sol. (ml)	Dose (ml)/ 100 g	No. of Insects in Jar	Mortality					Total Mortality %
			24 h	48 h	72 h	96 h	120 h	
Control	-	20	0	0	1	0	0	5%
0.2	1	20	0	2	4	0	4	50%
0.4	1	20	4	3	6	0	0	65%
0.6	1	20	0	4	3	5	2	70%
0.8	1	20	6	2	6	2	0	80%
1.0	1	20	2	2	0	4	6	70%

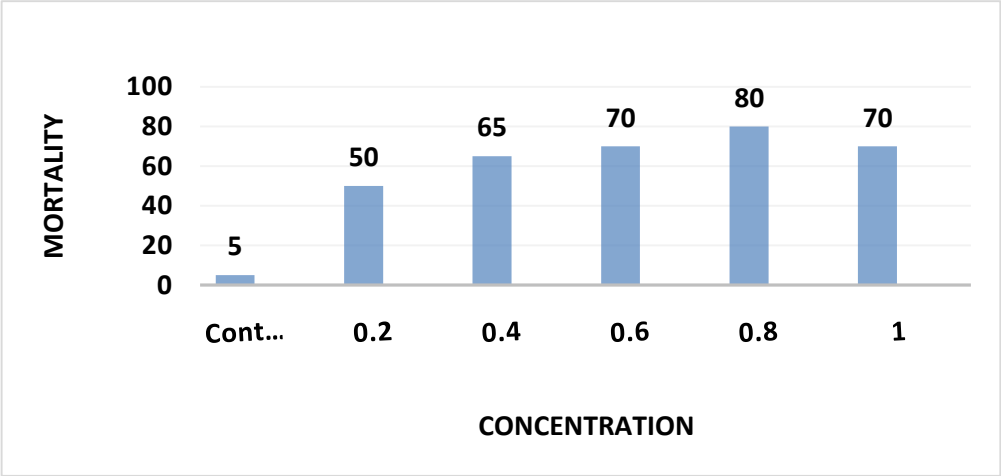

Fig. 1: Effect of Ethanol extract of *Tagetes erecta* (yellow flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolour.*

Results and Discussion

Sorghum (Sorghum bicolor) is an important cereal crop grown worldwide for food and animal feed purposes. Sorghum is a common pulse used as sprouted grains for several preparations of breakfast and meals in the country. It is highly susceptible to the infestation of the Sitophilus zeamais. S. zeamais larvae feed on the interior of individual grains, often leaving only the hulls, a flour-like grain dust, mixed with frass, is evident. The female chews through the surface of the grain, creating a hole. Infested grains contain holes through which adults have emerged. Like many other stored grain pests, Sitophilus species tolerate or prefer low moisture levels in their food.

The effect of extracts of *T. erecta* at different solvents showed variable damage to species *S. zeamais* (Tables 1-6; Figs. 1-6). It was observed that as the concentration of the working solution increases the per cent mortality of *S. zeamais* also increases, The per cent corrected mortality was negligible in the control of Aqueous, Ethanol and Butanol extracts.

Table 1 and Figure 1 illustrate the effect of Ethanol extract of *Tagetes erecta* (yellow flower) on grain pest, *Sitophilus zeamais* in Sorghum. The

Table 2: Effect of ethanol extract of *Tagetes erecta* (orange flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolor*

Conc. of Stock sol. (ml)	Dose (ml)/ 100 g	No. of Insects in Jar	Mortality					Total Mortality %
			24 h	48 h	72 h	96 h	120 h	
Control	-	20	0	0	1	0	0	5%
0.2	1	20	0	2	2	0	0	20%
0.4	1	20	4	0	3	6	0	65%
0.6	1	20	4	8	4	0	0	80%
0.8	1	20	3	5	5	3	4	100%
1.0	1	20	1	2	4	8	2	85%

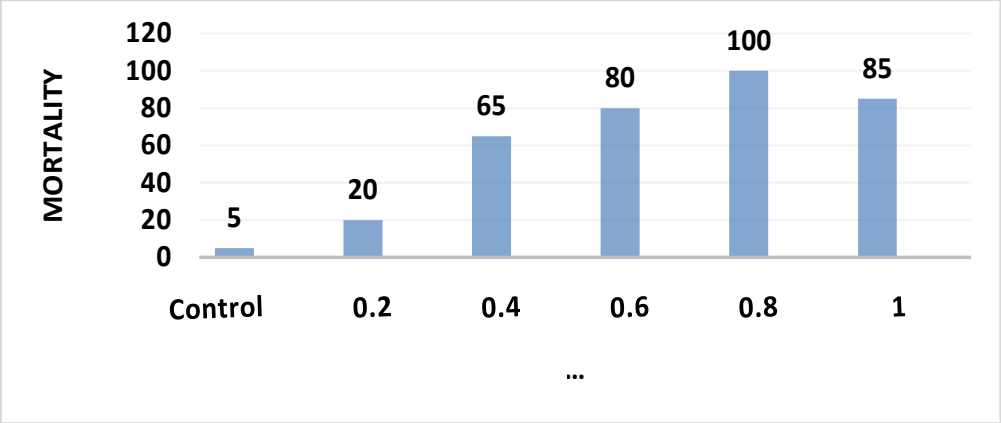

Fig. 2: Effect of Ethanol extract of *Tagetes erecta*(Orange flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolour.*

Table 3: Effect of Butanol extract of *Tagetes erecta* (yellow flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolor*

Conc. of Stock sol. (ml)	Dose (ml)/ 100 g	No. of Insects in Jar	Mortality					Total Mortality %
			24 h	48 h	72 h	96 h	120 h	
Control	-	20	0	0	0	1	1	10%
0.2	1	20	2	2	0	3	2	45%
0.4	1	20	0	0	2	2	0	20%
0.6	1	20	0	2	2	8	2	70%
0.8	1	20	2	1	3	3	2	55%
1.0	1	20	0	6	4	4	4	90%

highest 80% of mortality of *Sitophilus zeamais* was observed at 0.8 ml concentration of extract as shown in Figure 1. Table 2 and Figure 2 depict the effect of Ethanol extract of *Tagetes erecta* (Orange flower) on grain pest, *Sitophilus zeamais* in Sorghum. The 80% and 100% mortality of *S. zeamais* were observed against 0.6 and 0.8 ml concentrations of the stock solution, respectively (Fig. 2).

Table 3 and Figure 3 depicts the effect of Butanol extract of *Tagetes erecta* (yellow flower) on grain pest, *Sitophilus zeamais* in Sorghum. 90%

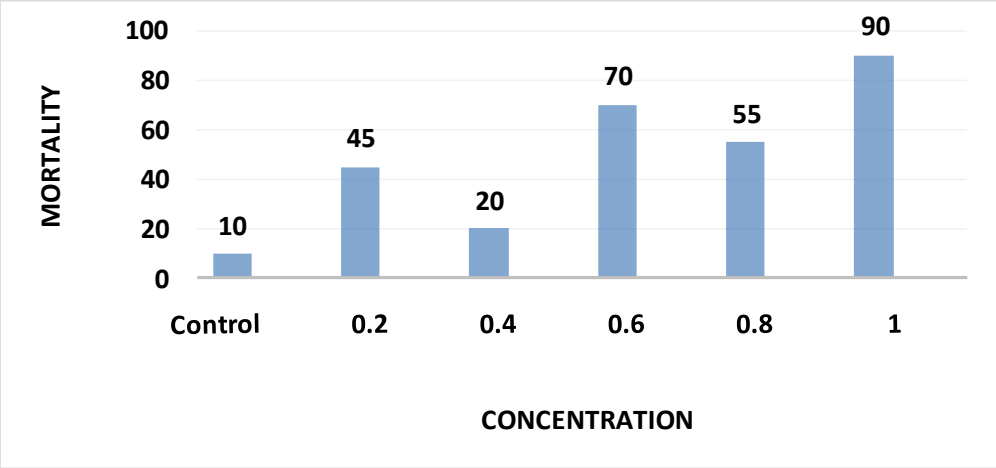

Fig. 3: Effect of Butanol extract of *Tageteserecta* (yellow flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolour.*

Table 4: Effect of butanol extract of *Tagetes erecta* (Orange flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolor*

Conc. of Stock sol. (ml)	Dose (ml)/ 100 g	No. of Insects in Jar	Mortality					Total Mortality %
			24 h	48 h	72 h	96 h	120 h	
Control	-	20	0	0	0	1	1	10%
0.2	1	20	0	2	2	2	0	30%
0.4	1	20	0	2	0	2	4	40%
0.6	1	20	2	0	1	4	2	45%
0.8	1	20	1	4	2	0	2	45%
1.0	1	20	2	2	0	6	2	60%

mortality was observed at 1 ml concentrations of the stock solution as shown in Figure 3.

Table 4 and Figure 4 depict the effect of Butanol extract of *Tagetes erecta* (orange flower) on grain pest, *Sitophilus zeamais* in Sorghum. Only 60% of mortality was observed in this against 1 ml concentration of stock solution as shown in Figure 4.

Table 5 and Figure 5 illustrates the effect of Aqueous extract of *Tagetes erecta* (yellow flower) on grain pest, *Sitophilus zeamais* in Sorghum. 45% of mortality was observed against 1 ml of stock solution as shown in Figure 5.

Table 6 and Figure 6 depict the effect of Aqueous extract of *Tagetes erecta* (orange flower) on grain pest, *Sitophilus zeamais* in Sorghum. Only 30% of mortality of *Sitophilus zeamais* at 1 ml was observed (Fig. 6).

Different parts of *Tagetes erecta* plant including flowers are used as a traditional medication to cure various illnesses. Leaves of this plant are used as antiseptic, in kidney problems, muscular pain, and piles. The flower petals are useful in fevers, epileptic suits (Ayurveda), astringent, carminative, stomachic, scabies, and liver complaints and are also employed in diseases

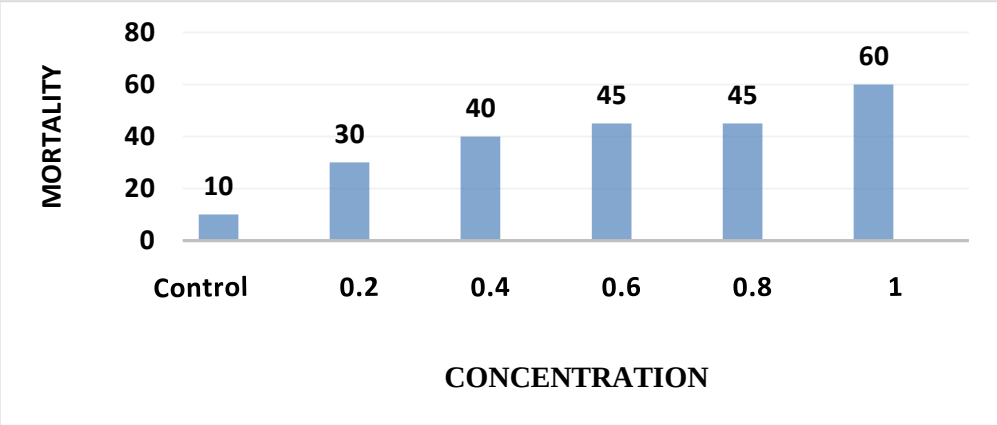

Fig. 4: Effect of butanol extract of *Tagetes erecta* (Orange flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolour.*

Table 5: Effect of Aqueous extract of *Tagetes erecta* (yellow flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolor*

Conc. of Stock sol. (ml)	Dose (ml)/ 100 g	No. of Insects in Jar	Mortality					Total Mortality %
			24 h	48 h	72 h	96 h	120 h	
Control	-	20	0	0	0	1	0	5%
0.2	1	20	0	0	2	2	0	20%
0.4	1	20	0	0	2	0	2	20%
0.6	1	20	0	2	1	2	0	25%
0.8	1	20	2	2	0	0	2	30%
1.0	1	20	2	0	4	0	3	45%

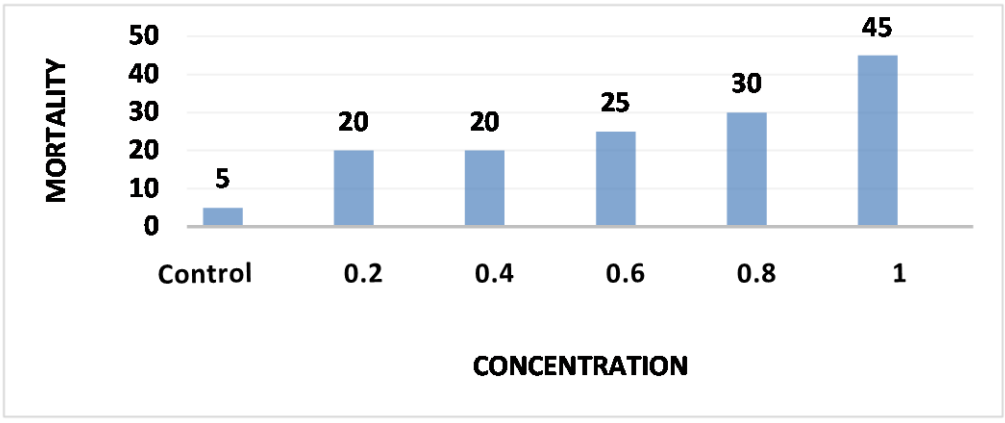

Fig. 5: Effect of Aqueous extract of *Tagetes erecta* (yellow flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolour.*

Table 6: Effect of Aqueous extract of *Tagetes erecta* (Orange flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolor*

Conc. of Stock sol. (ml)	Dose (ml)/ 100 g	No. of Insects in Jar	Mortality					Total Mortality %
			24 h	48 h	72 h	96 h	120 h	
Control	-	20	0	0	0	1	0	5%
0.2	1	20	0	0	4	0	0	20%
0.4	1	20	0	2	0	2	2	30%
0.6	1	20	2	0	1	2	0	25%
0.8	1	20	2	4	0	0	0	30%
1.0	1	20	3	0	0	3	0	30%

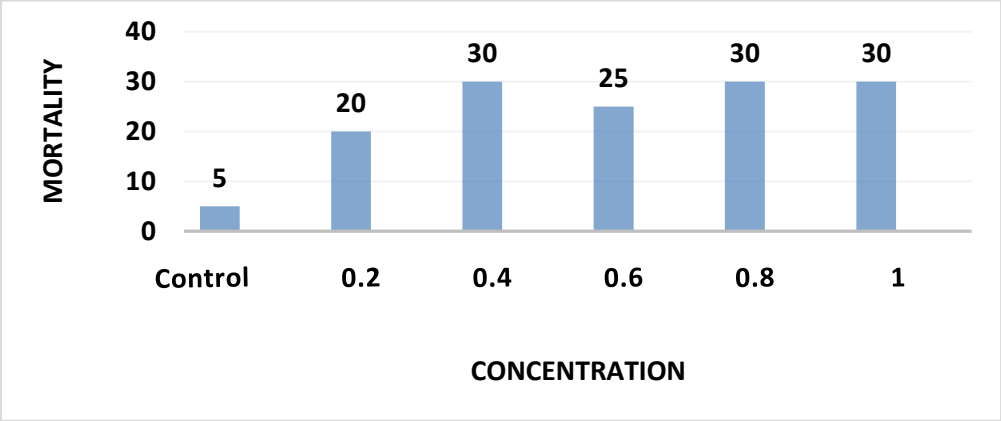

Fig.6: Effect of Aqueous extract of *Tagetes erecta* (Orange flower) on grain pest, *Sitophilus zeamais* in *Sorghum bicolour.*

of the eyes. They may be used to purify the blood and flower juice is given as a treatment for bleeding piles and also used in rheumatism, colds, and bronchitis (Rhama and Madhavan, 2011). The antibacterial activity of different solvents of *Tagetes erecta* flowers has been reported against *Alcaligens faecalis*, *Bacillus cereus*, *Campylobacter coli*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus mutants* and *Streptococcus pyogenes*. The flavonoid possesses antibacterial activity towards all the tested strains and indicates a maximum region of inhibition for *Klebsiella pneumonia* (29.50 mm). Flavonoid-patulitrin is one of the potential elements for its anti-bacterial activity (Kishore and Dwivedi, 2006). Fungitoxic activity of the essential oil of leaves of *Tagetes erecta* exhibited complete inhibition of the growth of *Pythium aphanidermatum*, the damping-off pathogen, at a concentration of 2000 ppm (Rodda *et al*., 2011).

Hydro-alcoholic extract of *Tagetes erecta* has been studied for its anti-diabetic activity. Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg b.w). It was found that the administration of *Tagetes erecta* extracts increased the glucose level seen after 30 min and the hypoglycemic impact was observed only after 120 min (Nikkon *et al*., 2011). The mosquitocidal effect of ethanolic extract of flowers of *Tagetes erecta* and its chloroform and petroleum ether soluble fractions towards the larvae of *Culex quinquefasiatus* were investigated (Prakash and Rao, 2018).

For managing stored produced insects, it is more secure to use plant materials with Insecticidal, anti-feedant, or repellent residences than the use of artificial insecticides (Allotey and Azalelor, 2000). Numerous plant species have been stated for these purposes in pest management (Andhale, 2022a). Herbal compounds of plant origin are biodegradable, frequently of low mammalian toxicity, and pose a low danger to the surroundings if used in small amounts. Recent research has focused on herbal product options for pest management in developing countries (Verma, 2018).

Plants belonging to the family Asteraceae had been significantly screened/studied for their larvicidal activity. The larvicidal activity of the crude flower and aerial extracts of Asteraceae plants, *Tagetes erecta* were studied towards *Culex quinquefasciatus*. The crude petroleum ether extract of plant extracts was effective (Salinas-Sánchez *et al*., 2012). *T. erecta* are poisonous to *Spodoptera frugiperda* larvae with massive toxic effects at the pupal level (Camaroti *et al*., 2018).

Andhale (2022b) has shown that the nutritive values of food are resolved by the development of leaves extracted from *Eucalyptus globulus* (Nilgiri) to targets of *Callosobruchus aculatus*. Formulation results show that the leaf extract of *Syzygigum cumini* (Jambhul) targets *Callosobruchus maculatus* to reserve the food grains (Andhale, 2022a).

The present study investigated the effects of Ethanol, Butanol, and Aqueous extracts of flowers of *Tagetes erecta* on *Sitophilus zeamais*. It was observed that the extracts of plant material, *Tagetes erecta* is effective against the growth and survival of *S. zeamais* in stored food grains as Sorghum. *S. zeamais* infested not only Sorgum but also maize, wheat, and rice. The increase of concentration of the formulated extract of *T. erecta* was directly proportional to the mortality rate of *S. zeamais*. *T. erecta* have medicinal values which will get added to *Sorghum bicolor* while preservation strategies using this kind of biopesticides. This may boost the immune of an individual and has no side effects after using such food commodities.

Conclusion

The biopesticide formulation of flowers from *Tagetes erecta* in butanol and ethanol have shown adverse affects against *Sitophilus zeamais* that infested domestic food grains *Sorghum bicolor.* Aqueous extract of flowers of this plant has shown comparatively less effective to *Sitophilus zeamais* than that of butanol and ethanol. The concentration of extract of *Tagetes erecta* was shown directly proportional to the mortality rate of *S. zeamais*. Hence, extracts of plant materials *Tagetes erecta* can be used at a large scale against the growth and survival of *S. zeamais* in stored food grains such as sorghum, maize, wheat, and rice and minimize the damage to acquire quality and economy.

Ethical Statement

The author confirms that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Zoology and Department of Biotechnology, MES's Nowrosjee Wadia College (Autonomous)(affiliated to Savitribai Phule Pune University), Pune, Maharashtra, India

Acknowledgements

I sincerely express my heartfelt gratitude towards Miss Pooja B. Avare for her valuable efforts in this research work. My deep appreciation goes to the Zoological Survey of India (ZSI), Akurdi, Pune, India, for the invaluable assistance in identifying the species *Sitophilus zeamais*.

Conflict of Interest

The authors declare no conflicts of interest.

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