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Efficacy of some Color Agents as Stored Product Protectants

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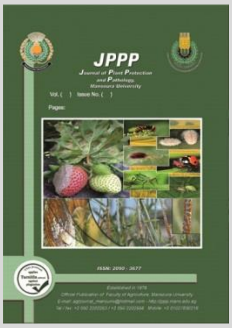

ABSTRACT

Cereals especially wheat grains are an important food source for the majority of the world population. Safe grain storage methods are thus of fundamental importance to ensure food supply from harvest to the next years. Consequently, the current study aimed to investigate the effect of non-customary application, seven color agents namely, brown chocolate, agricultural green, ponceau 4R., sunset yellow (FCF), tartrazine, titanium dioxide, and brilliant blue FCF on *Tribolium castaneum*, *Sitophilus oryzae* and *Trogoderma granarium* through measuring some of parameters: % mortality, effect on F_1 progeny, % weight loss and % germination compared to pirimiphos-methyl (as control). Results obtained indicated that percent of mortality for *S. oryzae* was between (13-58%) after 7 days which increased to (26.7-71.7%) after 12 days with the all tested coloring agents. Brilliant blue dye had the highest influence with LC_{50} values of 4.71 and 2.25% w/w after 7, 12 days, respectively. While, tartazine dye achieved the lowest action with LC_{50} values of 9.07 and 5.93% w/w at the same periods. For *T. castaneum* titanium dioxide had the highest impact with LC_{50} of 7.9 and 4.4% w/w, *T. granarium* had the highest response to brilliant blue (FCF) with LC_{50} of 5.24 and 1.1% w/w, after 7 and 12 days, respectively. The tested coloring agents reduced the population and reduced the percent weight loss compared to control. Eventually, the current study suggest using the tested colors as alternative control methods, however further studies to ensure their safety, as food additives, are needed.

Keywords: Pirimiphos-methyl, *S. oryzae*, *T. castaneum*, *T. granarium* and color agents.

INTRODUCTION

About 600 species of beetle pests attack stored products over the world (Rajendran, 2002). During storage periods, grains are destroyed by many stored grain insects that are responsible for world-wide up to 10-40% annually (Ahmed, *et al.*, 2013). The rice weevil, *Sitophilus oryzae* is of the most serious pests of cereal grain in Egypt and other countries. *Tribolium castaneum* is the most common and prevalent pest species of stored grain (Zettler and Cuperus, 1990). The khapra, beetle, *Trogoderma granarium* (Everts) is one of the most serious insect pests in tropical and sub-tropical regions of Asia and Africa (Atwal, 1976, Salunkhe *et al.*, 1985; and Viljoen, 1990). The use of synthetic pesticides on food materials posses many problems (Golb and Webely 1980).

Seed coloring is assuming importance and is being practiced by private seed companies mainly to enhance their company image and trade mark. Seed coloring offers industrial fronts. It improves the appearance of blended seeds, marketability and consumer preference. It combats storage diseases and pest and helps in reduction of storage losses of seeds. The carryover seeds can be easily identified by the color which will also help in taking prompt decisions in disposing of seeds (Navi *et al.*, 2006). It prevents adulteration of seeds. Ryker (1959) reported that dyes have no deleterious effects on seed storability. While, Tonapi (1989) obtained differential effect of dyes on seed quality in sorghum. Color preferences by economically important insects have been demonstrated by Prokopy and Owens (1983).

Giurfa and Menzel (1997) reported that insects have sophisticated visual abilities that allow them to scope efficacy with environment, more so the compound eyes of insects are adapted not only to detect motion, but also to see colors polarized light and geometric patterns.

Additionally, visitor insects may be interested in the plant or plant materials because of colors shapes. Monitoring traps aid in control of Coleoptera storage pests have been developed for both comerchial use in large scale industry in developed countries and for small scale use amongst subsistence farmers in developing countries (Barak and Burkholdr, 1985; Collins and Chambrs, 2003; Ukeh *et al.*, 2008 and Campbell, 2012). Color vision in some insect species has been extensively studied especially for pollinators, (Lunau and Maiet, 1995; Chittka, Raine, 2006). Blakmer *et al.*, (2006) and Demirel and Cranshaw (2006) have worked extensively on traps based on color characteristics in the field and greenhouse and reported that they are effective for controlling a variety of pests. Manueke *et al.*,(2015) studied performances of *S. oryzae* types and colors of storage containers.

Previous studies reported the presence of color preference or avoidance in *T. castaneum* grubs and adults (Ramose *et al.*, 1983; Viswan-than *et al.*, 1996 and Khan *et al.*, 1998; Hashem *et al.*, 2021). Moreover, Arnold *et al.*, (2015) investigated the response to color and host odor cues in three cereal pest species. Coloring materials, whether natural or artificial, are used for many purposes, including coloring foodstuffs, medicines and cosmetics, as well as coloring the seeds of some plants. Numerous studies have been conducted on colorants, targeting their effect as an additive to food items on the health of children, women and men. Previous studies also targeted the effect of color materials on insects as attractive and repellent materials, as well as the effect of different colors of light and colored utensils as insect traps in order to predict infection rates and thus the possibility of compating these insects.

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By reviewing the published researches in the field of the effect of colorants on insects, we did not recently find anything related to the effect of their pernicious action on insects specially insects of grain and stored materials. Therefore the current study aimed to bridge this gab through investigate the effect of seven seed colors (dyes) namely, Brown chocolate, Agricultural green, Ponceau 4R., Sunset yellow (FCF), Tartrazine, Titaneum dioxide, and brilliant blue FCF on adult mortalities of three species of stored product insects, the rice weevil, *S. oryzae* (L.), the red flour beetle, *T. castaneum* (Herbst) and khapra beetle, *T. granarium* (Everts), the reduction of F_1 progeny, percent loss of grain weight and percent germination compared to the chemical insecticide pirpmpphos-methyl as a standard protectant against stored product insects.

MATERIALS AND METHODS

1.Insects cultures:

The three tested insects were reared and maintained at the laboratory of Stored Product Res. Dept., Plant Protection Institute, Sakha Agric Research Station.

The red flour beetle, *Tribolium castanenum* (Herbst) (Tenebrionidae) (Coleoptera).

Insects were reared on wheat grain mixed with wheat flour. Grain were cleaned and sterilized by heating at 70°C for one hour, and put in glass jar each containing 400 g (30% wheat flour) and provided with (100-200) adult insects. Jars were covered and placed under laboratory conditions of 30±2°C and 65±5% R.H. The newly emerged adults (1-2 weeks old) were used in the further tests.

Rice weevil, *Sitophilus oryzae* (L.) (Curculionidae) (Coleoptera)

The adults of rice weevil, *S. oryzae* were reared on wheat grains under the laboratory conditions of 26±1°C, 65±5% R.H. Insects were maintained in small glass jars, each contained 200 gm of wheat grains and 100-200 adult insects. Adults were left for two weeks for egg laying in the jars and were then removed. Two weeks later, insects were collected by sieving the culture using a 10-mesh brass sieve. Insects (1-2 week old) were collected to use in the further experiments.

2.Colouring agents:

Sunset yellow (FCF)

IUPAC name: Disodium 6-hydroxy-5-[(4-sulfophenyl) azo]-2-naphthalene sulfononate

Color index: (C.I.): 15985

E Number: (E110)

Molecular formula: $C_{16}H_{10}Na_2O_7S_2N_2$

Molar mass: 452.37 g/mol

Melting ppoint: 300°C

Standard state: (at 25°C, 100 KPa).

Tartrazine

IUPAC name: Trisodium (4E)-5-oxo-1(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)hydrazono]-3 pyrazolecarboxylate

Color index: (C.I.): 19140

E Number: (E102)

Molecular formula: $C_{16}H_9N_4Na_3O_9S_2$

Molar mass: 534.3 g/mol

Melting point: 300°C (Decomposes at 25°C)

Standard state: at (25°C, 100 KPa).

Poceau 4R

IUPAC name: Trisodium (8Z)-7-oxo-8-[(4- sulfonatophthlen-I-YL) hydrazinylidene] naphthalene-1,3-disulfonate.

Other name: RasBerx Red.

Color index: (C.I.): 16255

E Number: (E124)

Molecular formula: $C_{16}H_{11}N_2Na_3O_{10}S_3$

Molar mass: 604.47 g/mol

Melting point: No sharp melting point observed.

Standard state: (at 25°C, 100 KPa).

Brilliant Blue FCF

IUPAC name: Ethyl-[4[4-(ethul-[3-sulfo phenyl) methyl] amino] phenyl]-(2-sulfophenyl)-methylidene]-1-cyclohexa-2.5-dienylidene)-[3-sulfophenyl)methyl] ammonium.

Color index: (C.I.): 42090

E Number: (E133)

Molecular formula: $C_{37}H_{34}N_2Na_2OgS_3$

Molar mass: 792.85 g/mol

Melting point: Decomposes at 283C withot melting.

Standard state: (at 25°C, 100 KPa).

Titanium dioxide

IUPAC name: titanium (IV) oxide

Color index: (C.I.): 77891

E Number: (E171)

Molecular formula: TiO_2

Molar mass: 1843

Standard state (at 25°C, 100 KPa).

Brown chocolate

IUPAC name: 1,4-dinydroxy-3,5-di(4-Su)po-1-naptliylazo) benzyl alcohol disoldium 4.4-[2.4-di hydroxyl-5-(hudroxymethyl)-1.3-phenylene) bis (azol) bisnaphthalene-1-sulphonate.

Molecular formula: $C_6H_8N_2Na_2O_8S_2$

Molar mass: 466,35 g/mol

Contains:

1) Tartrazine 2) Brilliant FCF
2) Azorubine 3) Sunset yellow (FCF)

Agricultural green

Contains:

1) Brilliant Blue FCF 2) Tartrazine

3.Pesticides treatment:

Pirimiphos methyl

Trade name: Actellic 50% EC

Chemical name: 2-diethylamino-6-methyl-pyrimidin-4-dimethyl phosphorothionate

Empirical formula: $C_{11}H_{20}N_3O_3P_5$

Producer: Kafr El-Zayat company, Egypt.

Molecular weight: 305.3

Formulation: 50% emulsifiable concentrate (EC).

4.Bioassay application method

Colour agents:

Samples of 20 gm of wheat grain or cowpea seeds were mixed thoroughly with four different concentrations, 0.5, 2, 4 and 6 % w/w. 20 adults *T. castaneum* and *S. oryzae* and *T. granarium* (1-2 week) were introduced to each glass jar (10 x 3 cm) containing treated medium.

The jars were covered with muslin cloth and fixed with rubber bands. Every treatment and control were replicates three times. All jars were kept at 30±1°C and 65+5 R.H%.

Tested insecticide (Actellic)

Pirimiphos-methyi was used as a standard reference using the method of mixing with feeding medium. Batches of uninfested wheat grain (of moisture content 9%) were weighed and placed in wide-mouth glass jars. The insecticide was diluted in water and added to the grains at rates which give the required concentration.

Jars were mechanically shaken for adequate and fixed time to ensure complete mixing process. Serial concentrations were made. The treated grain were allowed to dry at room temperature. For each concentration, twenty gram of treated grains were placed in a Petri dish (9 cm in diameter) and this was replicated four times. Ten adults of the tested insects *S. oryzae* and *T. castaneum* and *T. granarium* (1-2 weeks) were transferred to each dish. Mortality counts were recorded after 24 hours and corrected by Abbott's formula (1925). LC_{50} values were calculated by the method of Finney (1952).

5.Statistical analysis:

Data were analyzed using one way ANOVA and subjected to Duncan's multiple range test (1955).

Mortality

Mortality was assessed after 3, 5 and 7 days from application. Mortality data were corrected for control mortality using Abbott's correction formula:

$$\%CM = \frac{(\%T - \%C)}{(100 - \%C)} \times 100 \text{ (Abbott, 1925).}$$

Where:

CM = The corrected mortality T = The mortality in treated seed
C = the mortality in untreated seed
Concentration — mortality response lines were drawn
LC_{50} and slope values were calculated according to the method Finney (1952).
Control mortality did not exceed 10%

F1 progeny emergence

At the end of each specified period we removed the dead and lived insects from jars. Jars were covered with muslin kept in position with rubber bands and stored under laboratory conditions to allow insects to complete their-life cycle. At the end of life cycle period, the number of F_1 progeny was recorded every two days till (35-42) days for *T. castaneum* and (28-35) days for *S. oryzae* and *T. granarium*. Adults emerged were counted and reduction of progeny was calculated as follows:

$$\% \text{ Reduction} = [(C-T)/C] \times 100$$

Where: C = No. of adults emerged in control.
T = No. of adults emerged in treatment.

Weight loss:

The F_1 progeny population was assessed daily and removed up to a period of four weeks, the contents of each jar were sieved to remove the dusts, frass and any insect present in the grains. The weight of the grains was computed according to Harris and Lindblad (1978).

$$\% \text{ wt loss} = (W_i - W_f)\ 100/wi$$

Where: W_i = Initial weight W_f = Final weight

Seed germination

In order to assess the viability of seeds, seed germination was tested using 20 randomly picked seeds from undamaged grains after separation of damaged and undamaged grains in each jar. The seeds were placed on a moistened filter paper in plastic Petri dishes and the number of germinated seeds was recorded after 10 days.

RESULTS AND DISCUSSION

Color Additives History

A color additive, as defined by regulation, is any dye, pigment, or other substance that can impart color to a food, drug, or cosmetic or to the human body. Color additives are important components of many products, making them attractive, appealing, appetizing, and informative. Added color serves as a kind of code that allows us to identify products on sight, like candy flavors, medicine dosages, and left or right contact lenses. One of the U.S. Food and Drug Administration's (FDA) roles is to assure that color additives are safely and appropriately used.

Color additives are classified as straight colors, lakes, and mixtures. Straight colors are color additives that have not been mixed or chemically reacted with any other substance (for example, FD&C Blue No. 1 or Blue 1). Lakes are formed by chemically reacting straight colors with precipitants and substrata (for example, Blue 1 Lake). Lakes for food use must be made from certified batches of straight colors. (One exception is carmine, which is a lake made from cochineal extract.) Lakes for food use are made with aluminum cation as the precipitant and aluminum hydroxide as the substratum. Mixtures are color additives formed by mixing one color additive with one or more other color additives or non-colored diluents, without a chemical reaction (for example, food inks used to mark confectionery).

"Color" includes white, black, and gray. In addition, any chemical that reacts with another substance and causes formation of a color may be a color additive. For example, dihydroxyacetone (DHA), when applied to the skin, reacts with the protein of the skin to impart color. Even though DHA is colorless, it acts as a color additive when used for this purpose and is regulated as a color additive.

There is no "generally recognized as safe" (GRAS) exemption to the definition of a color additive. The Federal Food, Drug, and Cosmetic Act (FD&C Act) provides that a substance that imparts color is a color additive and is subject to premarket approval requirements unless the substance is used solely for a purpose other than coloring.

Naturally occurring color additives from vegetable and mineral sources were used to color foods, drugs, and cosmetics in ancient times. Paprika, turmeric, saffron, iron and lead oxides, and copper sulfate are some examples. The early Egyptians used artificial colors in cosmetics and hair dyes. Wine was artificially colored beginning in at least 300 BC. (Barrow *et al.*, 2003).

1. Effect of colouring agents:

Abo Arab and Salem (2018), assessed the repel activity of six colors beside one uncolour as control against *R. dominica* (Fabricious), *S. oryzae* (L) and *T. castaneum* (Herbest). They found that, response of insects significantly differed in terms of preference for the studied colors. Result presented that some colors caused high percent of inhibition against *R. dominica* with F_1 reduction percentages value reached above 80%. They suggested use of these colour as safe alternatives to chemical insecticides. Abo Arab and El-Tawelh (2015), reported that the all tested colours, blue, black, brown, green, orange, red and yellow influenced the orientation behavior of the tested insects and reduced the F_1 progeny of *R. dominica* and *S. oryzae.*

Natural dyestuffs produced by plants and insects have been used not only for dyeing silk but also so colouring agents in food and cosmetic industries. The interest in these natural dyestuffs is increasing because of recently discovered useful functions such as antioxidant effect (Yamazaku, 2002) and antibacterial effects (Kato *et al.*, 2004), in addition to the positive feeling people have about their safety. They have also gained popularity for the sober and elegant shades that they give to fabrics. It is traditionally believed that many of these natural dyestuffs are effective against insect attack and have some medicinal value.

Consequently, laboratory experiments were adopted in Department of Stored Product Insects, Sakha Agric. Res. Station to evaluate some coloring agents purchased from the local market as grain protectants against three of stored products insects; *S. oryzae*, *T. castaneum* and *T. granarium*. The present experiments aimed to estimate the percent of mortality, number of adult emergence, percentage reduction of progeny and the percentage loss of wheat grains, beside the permination.

Effect on mortality:

Tested colour dyes solution were prepared by dissolving the dyes in required amount of water. Seeds (cleaned and sterilized) were treated by dye solution separately using separate jars rotating slowly manually to ensure uniform coating of the colour. Four concentrations of colour dye each were used which ranged from 0.5% to 6.0% wt/wt.

Results summarized in Tables (1-3) showed that the all tested dyes, brown chocolate, agricultural green, ponceau, sunset yellow, tartazine, titanium and brilliant had moderately percent of mortality which ranged from 13.40% to 58.40% for *S. oryzae* adults (Table 1), 11-52% for *T. castaneum* (Table 2) and 11.60% to 56% for *T. granarium* (Table 3) at the all tested levels of the evaluated dyes after 7 days of exposure periods. Percent of mortality increased to 25 to 71.7%, 13-61% and 18.3-70.21% after 12 days post treatment for the three tested insects, *T. castaneum*, *S. oryzae* and *T. granarium*, respectively (Table 1-3) at all the levels of concentrations of the all tested dyes. Results obtained in Table (1) cleared that brown chocolate had the highest influence with LC_{50} of 5.56 and 2.76 wt/wt against *S. oryzae* adults while Ponceau 4R achieved the lowest action after 7 and 12 days of treatment with LC_{50} of 7.20 & 5.38 wt/wt.

Results in Table (2) showed that agriculture green dye had the lowest effect among the seven tested dyes with 11.0 and 13% mortality while the titanium dye was the effective one with 52 & 61% mortality of *T. castaneum* adults after 7 and 12 days of treatment. The rank of the dyes tested had the descending order following titanium, panceau.

Brown chocolate, sunset yellow, tartazine, brilliant and agricultural green after 7 and 12 days of treatment. Based on LC_{50} results obtained in Table (3) cleared that the brilliant dye had the superior effect with LC_{50} of 5.24 & 1.1 followed by titanium, brown chocolate, agricultural green, tartazine, ponceau and sunset yellow dye, respectively against *T. granarium* adults 7 and 12 days of treatment.

Table 1. Effect of colouring agents on mortality of *S. oryzae*, population build up and weight loss of wheat grain

Colour	Conc. w/w	% mortality 7 d	% mortality 12 d	LC_{50} 7 d	LC_{50} 12 d	F_1	Reduc. %	Loss %
Brown chocolate	0.5	26.70	26.7	5.56	2.76	97 a-e	13.91	19.22 a-e**
	2	35.70	36.00			92 a-e	20.00	14.55 a-d**
	4	43.40	52.00			72 a-d	36.52	13.22 a-d**
	6	55.00	71.70			63 ab**	45.21	11.40 a-d**
Agriculture green	0.5	23.40	26.20	6.45	3.36	98 a-e	14.76	22.40 cde**
	2	33.30	34.02			87 a-e	24.34	18.50 a-e**
	4	41.70	52.00			82 a-e*	28.69	12.22 a-d**
	6	52.80	64.20			70 a-d**	39.13	11.40 a-d**
Ponceau 4R	0.5	13.40	25.00	7.20	5.38	103 cde	10.43	23.90 de**
	2	28.30	33.40			95 a-e	17.40	20.0 a-e**
	4	33.70	48.40			79 a-e*	31.34	16.40 a-e**
	6	52.40	52.30			70 a-d**	39.13	18.64 ef
Sunset yellow (FCF)	0.5	13.40	36.70	5.08	2.32	92 a-e	20.00	17.55 a-e**
	2	32.30	48.30			82 a-e*	28.69	16.64 a-e**
	4	46.70	50.00			77 a-e*	33.04	10.66 a-d**
	6	52.40	63.40			64 abc**	45.21	8.11 a**
Tartrazine	0.5	18.40	27.40	9.07	5.93	107 de	6.08	30.43 ef
	2	26.70	33.40			94 a-e	17.39	29.00 ef**
	4	35.40	45.00			85 a-e	26.08	22.00 b-e**
	6	49.70	53.99			78 a-e*	31.30	18.40 a-e**
Titanium dioxide	0.5	22.70	34.00	5.22	2.37	102 b-e	11.30	18.99 a-e**
	2	36.60	45.00			86 a-e	25.21	15.40 a-d**
	4	43.30	52.00			74 a-d**	35.65	12.06 a-d**
	6	55.00	66.00			60 a**	47.82	8.40 a**
Brilliant blue (FCF)	0.5	18.20	30.00	4.71	2.25	90 a-e	21.73	16.70 a-e**
	2	28.31	46.60			83 a-e*	27.82	11.55 a-d**
	4	46.70	60.00			72 a-d**	37.39	9.44 abc**
	6	58.40	63.70			60 a**	47.82	8.88 ab**
Control		0.0	0.0			115 e		38.33 f

Effect on population build up:

The effect of colouring agents tested on population build up of *S. oryzae*, *T. castaneum* and *T. granarium* was evaluated by exposing the required of the tested insects to dyes treated seeds, after different periods of treatment, the F_1 progeny starts to emerge and the produced numbers were recorded till the end of experiment. The all tested coloring materials reduced the population build up of the all tested insects compared to control. Percent reduction of the emerged adults ranged from 6.08 to 47.82 for *S. oryzae,* 14.28 to 71.94 for *T. castaneum* and 14.28 to 78.57 for *T. granarium* at the levels of all tested concentrations of the investigated dyes. Results cleared that percent reduction of F_1 progeny accompanied with the insecticidal activity of the tested dyes where the strongest dye (based on LC_{50}) had the highest reducing in F_1 progeny's (Tables 1-3).According to these results, the *T. granarium* was more susceptible followed by *T. castaneum* and *S. oryzae* based on the reduction of F_1 progeny.

Effect on weight loss:

After three months of treatment the dyed wheat grain were weighed and the % loss was calculated according to the following equation:

$$\frac{\text{Dry wheat weight (before treatment)} - \text{dry weight (after treatment)}}{\text{Dry weight (before treatment)}} \times 100$$

Results obtained in Tables (1-3) cleared depicted that the investigated colours significantly reduced the percent weight loss of wheat grain treated with dyes compared to control where the percent weight loss ranged from 17.30% with the lowest concentration of sunset yellow dye to 2% loss with the highest concentration of brilliant dye against 35.70% loss with the control of *T. granarium*. For *S. oryzae* the percent loss ranged from 30.43% at the lowest concentration of tartazine dye to 8.11% at the highest concentration of sunset yellow dye compared to control which had 38.33% weight loss. *T. castaneum* caused loss percent ranged from 11.39 to 26.70% in colour treated wheat grain.

Finally, colouring dyes are likely to be stored products protectants according to the results obtained where all the tested dyes reduced F_1 progeny, weight loss compared to control and achieved moderate mortality for the tested insects.

Table 2 .Effect of colouring agents on mortality of *T. castaneum*, population build up and weight loss of wheat grain

Colour	Conc. w/w	% mortality		LC_{50}		F_1	Reduc. %	Loss %
		7 d	12 d	7 d	12 d			
Brown chocolate	0.5	20.0	25	15.4	8.9	11 d-j**	27.27	21.36 g
	2	27.0	34			99 c-h**	35.25	19.45 hi
	4	35.0	40			83 bcd**	46.34	16.35 m
	6	43.0	49			65 ab**	57.27**	14.54 n
Agriculture green	0.5	11.0	13	25.77	13.3	132 jk	14.28	26.70 n
	2	18.7	20			129 ijk*	15.97	20.40 g
	4	29.0	31			119 f-j**	22.57	19.83 h
	6	33.0	40			95 c-g**	38.09	13.14 o
Ponceau 4R	0.5	20.0	28	13.00	5.0	92 c-i**	35.42	20.40 a
	2	28.0	35			89 b-f**	41.56	19.20 i
	4	35.0	42			85 bcd**	44.37	16.30 m
	6	45.0	59			82 abc**	46.46	12.40 p
Sunset yellow (FCF)	0.5	18.0	30	18.54	10.0	112 d-j**	27.05	23.52 c
	2	29.0	31			103 c-j**	32.83	19.07 i
	4	33.0	38			100 c-h**	34.93	17.41 l
	6	40.0	52			84 bcd**	45.16	13.50 o
Tartrazine	0.5	19.0	22	19.50	11.0	115 e-j**	25.19	23.52 c
	2	23.0	30			112 d-j**	26.84	18.07 k
	4	35.0	40			97 c-h**	36.79	17.37 L
	6	40.0	45			89 b-e**	71.94	13.47 o
Titanium dioxide	0.5	20.0	28	7.9	4.4	101 b-f**	34.13	20.33 g
	2	30.0	36			92 b-f**	40.00	18.13 k
	4	36.0	43			86 b-e**	43.86	16.23 m
	6	52.0	61			53 a**	65.29	11.93 q
Brilliant blue (FCF)	0.5	11.0	17	23.8	9.3	125 hij*	18.83	22.60 d
	2	19.0	28			123 g-j*	20.12	22.00 e
	4	26.0	35			111 d-j**	27.59	18.59 j
	6	36.0	48			93 b-f**	39.35	14.40 n
Control						154 k		29.44 a

Table 3. Effect of colouring agents on mortality of *T. granarium*, population build up and weight loss of wheat grain

Colour	Conc. w/w	% mortality		LC_{50}		F_1	Reduc. %	Loss %
		7 d	12 d	7 d	12 d			
Brown chocolate	0.5	26.0 32.0	25.0	13.22	5.42	83 ef**	46.10	14.35 i-m**
	2	39.0	35.0			67 ef**	56.49	10.25 d-i**
	4	45.9	46.0			52 abc**	66.23	8.10 b-f**
	6		53.3			43 abc**	72.07	4.89 ab**
Agriculture green	0.5	18.3	18.3	14.75	8.22	121 hi**	21.42	14.75 j-m**
	2	20.0	23.4			95 f**	38.31	12.70 g-l**
	4	35.0	36.6			88 ef**	42.85	8.30 b-f**
	6	43.3	51.5			55 bcd**	64.28	6.70 bcd**
Ponceau 4R	0.5	15.0	23.0	20.69	7.35	124 i**	19.48	15.75 Lm**
	2	21.6	35.0			102 fgh**	33.76	13.56 n-m**
	4	28.3	43.0			86 ef**	44.15	11.00 f-i**
	6	40.0	48.4			60 cd**	61.03	8.00 b-f**
Sunset yellow (FCF)	0.5	11.6	28.4	23.49	8.85	132 i*	14.28	17.30 m**
	2	16.6	33.0			122 i*	20.77	15.00 kLm**
	4	23.3	41.7			100 ni**	35.06	12.85 g-l**
	6	36.3	50.0			74 de**	51.94	7.5 b-e**
Tartrazine	0.5	19	20.6	16.52	5.09	115 ghi**	29.22	13.5 n-m**
	2	25	28.6			87 ef**	43.50	11.4 e-k**
	4	35	38.0			61 ed**	60.38	9.69 c-h**
	6	41	63.3			44 abc**	71.42	505 abc**
Titanium dioxide	0.5	28.0	38.0	7.05	1.95	92 ef**	40.25	12.32 f-L**
	2	38.1	42.0			62 cd**	59.74	10.55 d-j**
	4	43.4	56.0			55cd**	64.28	8.60 b-g**
	6	50.2	70.0			38 ab**	75.32	5.40 ab**
Brilliant blue (FCF)	0.5	29.7	41.6	5.24	1.10	88 ef**	42.85	11.99 f-L**
	2	34.8	56.6			53 abc**	64.28	8.99 b-g**
	4	45.3	63.4			52 abc**	66.23	6.87 bcd**
	6	56.0	70.21			33 a**	78.57	2.00 a**
Control						154 j		35.7 n

Effect on germination:

The present results demonstrated that except some low concentrations of the tested colouring agents, the all tested agents nearly had harmful effect on germination percent with the three tested insects despite the tested agents achieved moderately mortality percent against these insects.

These results mean that the harmful effect on germination % due to the tested colour agents (Tables 4-6). Also, the dyes differed significantly among themselves for germination percent (Tables 4-6).

Ryker (1959) reported that dyes have no deleterious effects on seed storability. While, Tonapi (1989) obtained differential effect of dyes on seed quality in sorghum. Navi *et al.* (2006) evaluated the influence of seed colour with and without insecticide on rice weevil (*S. oryzae*) incidence during storability of hybrid sorghum. They tested three colour; red, yellow and green with and without malathion. They found that green colour + malathion (h/kg of seed) had showed the highest germination percent throughout the observation period compared to control and chemical alone. Minimum infestation due to insect was noticed in green colour.

Also, seed coulour with insecticide had recorded higher germinability compared to colour only. Only coloured seeds showed minimum infestation compared to untreated check. Kato *et al.,* (2004) studied damage to wool fabrics dyed with different natural and chemical dye stuffs by the larvae of vared carpet beetle, *Anthrenus verbasci*. Eight of ten natural dye stuffs showed an antifeeding effect against *A. verbasci*. They also reported that the damage to dyed fabrics by the insect was not related to the extent of colour depth or shade of the dyed fabrics.

Table 4. Effect of colouring agents on germination of wheat grain exposing *S. oryzae* adults.

Colour	Conc. w/w	Germination %
Brown chocolate	0.5	93.00 hij
	2	92.00 ghi*
	4	89.30 e-h**
	6	87.00 cde**
Agriculture green	0.5	92.00 ghi*
	2	89.00 d-g**
	4	83.00 ab**
	6	81.00 a**
Ponceau 4R	0.5	91.00 c-f**
	2	89.30 e-h**
	4	89.00 d-g**
	6	87.00 cde
Sunset yellow (FCF)	0.5	92.00 ghi*
	2	89.50 e-h**
	4	87.00 cde**
	6	85.00 bc**
Tartrazine	0.5	93.00 hij
	2	91.00 fgh**
	4	87.60 c-f**
	6	84.70 abc**
Titanium dioxide	0.5	95.00 ij
	2	93.00 hij
	4	91.00 fgh**
	6	87.00 cde**
Brilliant blue (FCF)	0.5	89.00 d-g**
	2	87.00 cde**
	4	85.40 bcd**
	6	82.00 ab**
Control		96.00 j

Agnieszka and Houbowicz (2008) studied the effect of pansy seeds colour (yellow, brown and dark) on their germination, they found that there was a clear effect of seed colour on the seeds germination. The dark seeds had the highest germination capacity, whereas the yellow ones had the lowest. The experiments proved that color sorting of pansy seeds can improve their germination mostly due to eliminating light, immature seeds.

Sawarderkar *et al.* (2008) soaked cucumber seeds for 5 minutes in a colouring solution (red, blue, yellow or green) with or without 2% Bavistin (carbendazim), then stored in polyethyelene nags at ambient temperature for 12 months. They found that the minimum germination percentage (80%) was maintained for up to 12 months in all treatments. They also found that the increase in vigour index for red seeds with Bavistin was due to greater germination and root and shoot lengths, as well as lower infection rate.

Tonapi *et al.* (2006) studied the effect of 25 dyes on the quality of rice (MTU7029 and BPT5204) and maize (Saranath and Deccan 101) seeds. Seeds were soaked in the dye solution (0.75% concentration) tor 3-5 minutes. The dyes had positive or deleterious effects on seed quality and seedling growth parameters.

Table 5. Effect of colouring agents on germination of wheat grain exposing *Tribolium castaneum* adults.

Colour	Conc. w/w	Germination %
Brown chocolate	0.5	91.00 kl**
	2	88.50 ij**
	4	86.00 fgh**
	6	84.50 efg**
Agriculture green	0.5	91.00 kl**
	2	87.00 ghi**
	4	84.00 def**
	6	82.00 cd**
Ponceau 4R	0.5	89.00 ijk**
	2	85.50 e-h**
	4	82.00 cd**
	6	79.00 ab**
Sunset yellow (FCF)	0.5	90.00 jk**
	2	85.00 e-h**
	4	82.00 cd**
	6	77.00 a**
Tartrazine	0.5	91.00 kl**
	2	90.00 jk**
	4	87.40 hi**
	6	83.00 de**
Titanium dioxide	0.5	93.00 L**
	2	91.00 kL**
	4	89.00 ijk**
	6	85.00 e-h**
Brilliant blue (FCF)	0.5	87.00 ghi**
	2	85.00 e-h**
	4	83.00 de**
	6	80.40 bc**
Control	96.00 m	

Keshavulu and Krishnasamy (2005) evaluated soybean seed coloured with botanical dyes namely flower extract of Hibiscus rosasinensis, root extract of beet root and rhizome extract of turmeric, synthetic chemical dyes (yellow, black and congored) and polykotes (yellow, black and red) at 100 ml/kg, 1% and 3 g (dissolved in 5 ml of water)/kg of seeds, respectively, for seed quality and bruchid (*Callosobnichus chinensis*) damage. The coloured seeds showed differences in quality and insect damage, the synthetic chemical dyes and red polykote protected the seeds from bruchid up to one month storage.

5. Efficacy of pirimiphos-methyl on tested insect species:

Results in Table (7 & 8) included the insecticidal activity of pirimiphos-methyl on mortality, percent reduction F_1 progeny of *T. granarium*, *T. castaneum* and *S. oryzae.*

Results obtained greatly explained that the chemical insecticide pirimiphos-methyl was the most effective agent against the three tested insect species compared to the all investigated materials (colouring agents) which used in the present study. Based on LC_{50} values pirimiphos-methyl achieved high percent mortality after 24 h of treatment with LC_{50} values of 2.41, 0.65, 1.41 and 0.55 mg/kg grain for *T. granarium, T. castaneum* and *S. oryzae,* respectively. *T. granarium* was the tolerant while *T. castaneum* was the susceptible one.

Table 6. Effect of colouring agents on germination of wheat grain exposing *Trogoderma* granarium adults.

Colour	Conc. w/w	Germination %
Brown chocolate	0.5	90.00 ij**
	2	87.00 gh**
	4	84.00 def**
	6	79.00 b**
Agriculture green	0.5	89.00 hi**
	2	87.00 gh**
	4	82.00 cd**
	6	78.00 b**
Ponceau 4R	0.5	87.00 fg**
	2	83.00 de**
	4	79.00 b**
	6	75.00 a**
Sunset yellow (FCF)	0.5	92.00 jk**
	2	90.00 ij**
	4	87.00 gh**
	6	85.00 efg**
Tartrazine	0.5	92.00 jk**
	2	90.00 ij**
	4	88.50 hi**
	6	85. efg**
Titanium dioxide	0.5	94.00 hl
	2	92.00 jk**
	4	90.00 ij**
	6	87.00 gh**
Brilliant blue (FCF)	0.5	87.00 gh**
	2	83.00 de**
	4	80.00 bc**
	6	78.00 b**
Control		96.00 L

Table 7. Toxicity of the chemical insecticide, pirimiphos-methyl against certain stored grain insects after 24 hr exposure to treated wheat grain.

Toxicant	Insect	LC_{50} (mg/kg)	Confidence limits		slope
			Lower	upper	
pirimiphos -methyl	*T. granarium*	2.41	2.107	2.721	2.717
	T. castaneum	0.65	0.301	0.920	2.276
	S. oryzae	1.41	1.155	1.640	2.757

Table 8. Effect of pirimiphos-methyl on mortality and reduction of F_1 progeny of *T. granarium*, *S. oryzae* and *T. castaneum* adults.

Insect	Conc. (%) w/v	% Adult mortality (24 h)	No. of F_1 progeny after 45 days	Reduction in F_1 progeny (%)
T. granarium	1.25	23.30	13.00 e	91.55
	2.50	50.00	6.00 cd	96.10
	5.00	80.00	2.00 abc	98.70
	10.00	93.30	0.00 a	100.00
Control	0.00	0.00	154 g	-
S. oryzae	1.25	46.66	8.00 d	93.04
	2.50	73.33	4.00 a-d	96.52
	5.00	96.60	0.00 a	100.00
	1.00	100.00	0.00 a	100.00
Control	0.00	0.00	115 f	-
T. castaneum	1.25	79.00	4.00 a-d	97.40
	2.50	88.00	2.00 abc	98.70
	5.00	99.99	0.00 a	100.00
	10.00	100.00	0.00 a	100.00
Control	0.00	0.00	154 g	-

CONCLUSION

From the present study the following aspects could be concluded:

1- Mortality percent of the tested insects increased with the increasing of concentrations and the period of exposure.
2- The rank of toxic action of the tested colouring dyes fluctuated with the different tested insects (based on LC_{50}).
3- The all tested dyes decreased the population build up and the weight loss of wheat grain.
4- The all tested dyes nearly declined the germination percent of wheat grain after one month of treatment.

It is known that the process of washing grain in mills is a common process through which it can reduce the potential side effects resulting from the coloring additives added to the grains or seeds. Accordingly, the current study recommends conducting the washing process before grinding the grains, as well as conducting further studies on insects on insects and other colored materials to confirm our results.

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كفاءة بعض المواد الملونة كواقيات للمواد المخزونة

رأفت بدر سعد أبوعرب ، ناريمان محمد عبدالسلام الطويلة ، جورج موريس نصر و أمل مصطفى حمزة

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة

تعتبر الحبوب النجيلية وخصوصاً القمح من أهم مصادر الغذاء للغالبية العظمي من السكان علي مستوي العالم. استخدام طرق وقاية آمنة للحبوب يعتبر من العوامل الأساسية الهامة في الحفاظ علي استمرار الامداد بالغذاء من بعد الحصاد وحتي العام القادم. سبب استخدام المبيدات الكيماوية في الوقاية من الآفات مشاكل كثيرة لذلك كان لابد من وجود طرق بديلة للمكافحة رخيصة وآمنة. استهدفت الدراسة الحالية –بناء علي ما سبق- دراسة تطبيق غير شائع يتمثل في اختبار كفاءة سبعة ملونات وهي: Brown chocolate، Agricultural green، Ponceau 4R، Sunset yellow (FCF)، Tartrazine، Titaneum dioxide، وأخيراً brilliant blue FCF ضد ثلاث حشرات وهي خنفساء الدقيق الصدئية الحمراء Rust red flour beetle (*T. castaneum*)، سوسة الارز *Sitophilus oryzae* وخنفساء الصعيد *Trogoderma granarium* من خلال بعض المعايير وهي النسبة المئوية للموت، والتأثير علي الخلفة في الجيل الأول، الفقد في الوزن وكذا التأثير علي نسبة الانبات. أظهرت النتائج المتحصل عليها أن جميع الملونات المستخدمة كان لها تأثير جيد علي المعايير المدروسة تحت التركيزات المستخدمة. أوضحت النتائج بالنسبة لسوسة الارز أن نسبة الموت تراوحت بين (13-58%) بعد سبعة ايام والتي زادت الي (26.7-71.7%) بعد 12 يوم من المعاملة مع كل الملونات المختبرة. أظهر brilliant blue أعلي نسبة تأثير حيث تراوحت قيمة الـ LC_{50} بين 4.71-2.25% وزن/وزن بعد 7، 12 يوم من المعاملة علي التوالي، بينما كانت صبغة الـ tartrazine هي الاقل تأثير مع LC_{50} تراوح بين 9.07-5.93% وزن/وزن عند نفس الفترات السابقة. اما بالنسبة لخنفساء الدقيق الصدئية *T. castaneum* فكان الـ titanium dioxide هو الاشد تأثرا (LC_{50}, 7.9-4.4% وزن/وزن) عند نفس فترات المعاملة السابقة. أظهرت خنفساء الصعيد *T. granarium* استجابة عالية لصبغة brilliant blue (FCF) (LC_{50}, وزن/وزن 5.24-1.1%) عند نفس فترات التعرض علي التوالي. أظهرت النتائج أن جميع المواد الملونة المستخدمة خفضت اعداد الذرية الناتجة في الجيل الأول للحشرات المختبرة مقارنة بالكنترول، بالإضافة الي ذلك كان هناك خفض معنوي في الفقد في الوزن مقارنة بالكنترول. أظهرت النتائج انخفاض طفيف في نسبة الانبات بعد شهر من المعاملة بالمواد الملوثة. كان المبيد الكيماوي الموصي به وهو البريميثوس-ميثيل pirimiphos-methyl هو الأشد تأثيراً علي جميع المعايير المدروسة مقارنة بالمواد الملونة. تقترح الدراسة الحالية استخدام المواد الملونة في المكافحة للحشرات المذكورة كأحد عوامل المكافحة المتكاملة بعد اجراء دراسات مستقبلية تؤكد أمان هذه الملونات علي الانسان والبيئة.